

H. B. Switzer

CLASSIFICATION OF DAIRY BACTERIA,

— BY —

H. W. CONN.

— . . . —

From Report of the Storrs (Connecticut) Agricultural Experiment
Station for 1899.

CLASSIFICATION OF DAIRY BACTERIA.

BY H. W. CONN.

COLLECTION OF THE BACTERIA.

For the last ten years, in which the work upon dairy bacteriology has been carried on for this Station, I have been gradually collecting from the dairy products of the vicinity a variety of forms of bacteria. These have been obtained from milk and cream and occasionally from butter. During that time a very large number of different cultures have been isolated from these dairy products, and have been studied in the laboratory by bacteriological methods with more or less completeness. Of the many hundreds thus collected, large numbers of course have proved to be duplicates. As fast, however, as it became apparent that any particular form isolated and studied was distinct from those previously characterized, the bacteriological characteristics of the new variety were carefully detailed and the form was entered in a list by number, together with the characteristics as they were determined. In this way there has accumulated a list of over 200 different types of bacteria which have been regarded as more or less distinct from each other. It may be assumed that this list contains probably all of the common species of bacteria which are likely to be found in dairies of this vicinity. Of this long list many of course have been found to be very commonly, some indeed almost universally, present in milk. Others are more uncommon, being found only a few times, and many indeed have been isolated only a single time and must therefore be regarded as purely accidental. It has been found, as would be expected, that the species of bacteria in a sample of milk vary somewhat with the locality from which the milk is obtained, and also with the season of the year in which the examination is made. A few forms of organisms are so widely distributed as to be almost universal, both as to locality and as to season.

Of the bacteria in my list there are quite a number that have not been described with sufficient accuracy to make the description of any considerable value. This is true especially of some

of the earlier varieties that were isolated before the methods of description were sufficiently worked out; and it is also true of some of the later ones which were by accident lost before a complete description was obtained. Moreover, during the period in which these experiments have been going on the methods of bacteriological work and description have been quite materially changed, and it is now considered essential to determine certain characteristics which in early years were not much attended to. The result is that the descriptions given beyond of the different bacteria are of varying value. No one can be more fully aware of the incompleteness of some of these descriptions than myself, and for this reason a considerable number of the bacteria in the list which I have collected will be left out from the following classifications because the descriptions are too incomplete to be of any particular value.

NEED OF CLASSIFICATION OF BACTERIA.

As the list of dairy bacteria in my hands has thus been increased, it has been found more and more necessary that some kind of classification and grouping of these different bacteria should be devised. When the number reaches into the scores it is a matter of very great difficulty to determine whether the new culture isolated from milk is really new or is identical with some of the forms previously studied, and it becomes therefore absolutely necessary that some simple means of grouping the different bacteria should be obtained to make this determination possible. During the past few years I have gradually developed a method of grouping these bacteria which has proved extremely useful in my laboratory and has very greatly simplified the problem of the further study of new varieties isolated.

Inasmuch as it has proved so useful in the determination of the forms found here, I have thought it not unwise definitely to formulate the method of grouping which has been used, and to publish along with it the descriptions of all of the more important of the varieties of bacteria which have been isolated here. There are at the present time a number of American bacteriologists working upon dairy bacteriology, and it is eminently desirable that they should be able to compare their results with each other. As long as the species found by one bacteriologist are described only in private notes, they cannot

of course be compared with those found by another, and the work of the different observers in different parts of the country cannot be brought into relation. It is certainly time that our dairy bacteriologists should begin to compare results. For the purpose of making a beginning in this direction, the following classifications and descriptions of bacteria have been put together and are now published. It is hoped that it may serve to enable the different dairy bacteriologists to compare the species of bacteria in one locality with those in another, and thus may aid in simplifying the problem of the species of dairy bacteria. Hitherto there has been nothing of this sort published in this country, nor indeed in Europe. Isolated descriptions of a few dairy bacteria have been published in one place and another, but no attempt has been made to get together under one list the types of bacteria which are found in dairy products. The water bacteria have been much more carefully studied and classified; and it is clearly a matter of importance that the dairy bacteria should in a similar way be brought under more or less distinct classification. The present list will therefore serve as a start in this direction.

METHOD OF CLASSIFICATION.

No one can be more fully aware of the incompleteness of the following list than I am myself. Strictly anaerobic species I have not yet studied, nor have I as yet made any study of the spore forming species which remain in milk after boiling. Some of the species are imperfectly described. Doubtless some of my species should be divided, and perhaps others that I have regarded as separate will subsequently be united into one. But all pioneer attempts at classifying bacteria must be open to criticism. It is my hope that this classification may be the beginning of a work which shall be slowly perfected, and may be at all events of use as a point of departure for the adoption of better plans in future. If it shall serve as a means of bringing together the work of American dairy bacteriologists its purpose will be accomplished.

Every one who has had anything to do with the descriptions of bacteria has been impressed with the difficulty of following through those given in the ordinary way and comparing them with each other. This is due partly to the great detail which is given in some cases and to the lack of detail in others. In

many of these descriptions nonessential features are given with great detail, and the whole becomes immensely confusing. It has seemed to me that the method adopted by Fuller and Johnson in a recent publication on water bacteria* marks a very decided advance in our method of arranging the characteristics of bacteria. They have devised a scheme whereby all of the important characteristics of a given species of bacteria may be briefly indicated by positive or negative signs, so that, by the proper arrangement of tables, it is possible by the use of a few of these signs to give in a very brief compass all of the important characteristics of the organisms to be described. The advantages of this scheme are manifold. In the first place it avoids the needless confusion of details which is so likely to arise from the verbose descriptions which may be given. In the second place it makes possible a direct comparison of species with each other, and enables one to determine at a glance whether two forms are in agreement so far as regards their chief characteristics. If then further details be given elsewhere, with more careful descriptions, the task of determining whether a new variety is identical with one already described is an easy one. The success of this method as applied to water bacteria has led me to adopt the same in the description and classification of the dairy bacteria, and the tables in the following pages are therefore based upon the same principle as those which have been used by Fuller and Johnson in their classification of water bacteria.

In these tables, however, I have found it necessary to adapt the plan to the descriptions which I have in my possession, and consequently to change the character of the table somewhat. In the study of dairy bacteria certain characteristics have been inevitably regarded as of more importance than others; and in the descriptions of the bacteria which I have been accumulating some factors, particularly those in connection with the action of the bacteria upon milk, have been studied in more detail than would be possible to indicate in the table of Fuller and Johnson. On the other hand, some of the characteristics which they have included in the study of water bacteria have not been determined at all, or only incidentally, in the case of the bacteria studied here. This is to be regretted, since it is eminently desirable that the different

* Jour. of Exp. Med., 1899.

bacteria should be comparable with each other. It is, however, at present unavoidable, because most of these species have not been preserved in culture in the laboratory, and it is now impossible to determine the characteristics which have not hitherto been made out. For these reasons the tables which I have been obliged to make out and to use differ in some details from those of Fuller and Johnson. I have, however, followed them as closely as possible.

SOURCES OF THE SPECIES.

A word as to the sources of the different species of bacteria which are here described. All have been obtained from dairy products. A majority of them have come from cream either from neighboring creameries or from private dairies, some being obtained from ripened cream, others from unripened cream. Some of them have been obtained from milk as delivered in Middletown by milkmen. Some have been obtained from the milk after it has stood in the pantries of private houses. Some of the species have been obtained directly from milk as drawn from the teats of the cow into sterilized vessels, others again from the dust which falls into the milk pails during the milking. Two of these species have been obtained from some samples of special milk which had been sent in cans from Uruguay; and some of the species have been obtained from samples of milk that were sent to Middletown from a large number of States in the Union, ranging from Maine to California. All, however, are strictly dairy organisms, being found in milk or its products.

METHOD OF ISOLATION AND STUDY.

The method of isolation and study requires little description, inasmuch as it has been, in general, that commonly used in bacteriological study. For the isolation of the bacteria from the milk ordinary gelatin has been used. In most of the early years the gelatin was made in the ordinary way, but in recent years it has been found that a much more satisfactory result is obtained if there is added to the gelatin three per cent. of milk sugar. The reason for this is manifest. Milk always contains a considerable portion of milk sugar. Naturally, therefore, it is to be expected that the typical dairy bacteria will grow much more rapidly in gelatin provided with milk sugar. Indeed, some

of the characteristic species are isolated with great difficulty from cream by the use of ordinary gelatin, but are found with the greatest of readiness by the use of sugar gelatin. Practically also it has been found extremely useful to employ for this purpose only gelatin which has been rendered blue with litmus solution, as this makes it possible readily to distinguish the acid organisms from those that do not produce acids. It has been found that one species of bacterium which is par excellence a dairy species, namely *B. acidi lactici*, No. 206, produces in such gelatin a colony which is most readily distinguished from any other species of bacteria, and this of course makes it extremely convenient for use.

After isolation of the bacteria they have been purified by replating in the customary manner, and then inoculated into the ordinary media for the purpose of determining their characteristics, which have been obtained as usual. Special attention, however, has always been given to the effect of the organisms upon milk at ordinary room temperature and at higher temperatures. In many cases also the action of the organisms upon sterilized cream has been determined, and in a considerable number of the cases the influence of the organisms upon the character of the butter obtained from cream ripened by means of them has been made out.

Unfortunately, the use of the fermentation tube has not been adopted so widely as would be desired. In the study of some species the fermentation tube has been used, but in the majority of cases it has not. In the work that is done now it is used in all cases, but in many of the descriptions which have been given in past years this important test was omitted. For this reason the production of gas in glucose bouillon has not been determined in many cases. In determining the characteristics of these bacteria no attempt has been made to determine their action upon nitrites or the production of indol, nor has any attempt to determine pathogenic characteristics been made. These features have not yet been regarded as of significance enough in the study of dairy bacteria for dairy purposes to warrant the time which would be taken in determining them.

GROUPING THE BACTERIA.

In dividing the dairy bacteria into groups I have tried so far as possible to follow the methods already adopted, and have

used as the foundation of my grouping that of Flügge given in the last edition of his *Die Microorganismen*. This grouping, as will be seen below, is based partly upon the power of producing pigment and partly upon morphological data. This arrangement is quite similar to that which has been adopted by Fuller and Johnson, and is easily compared with that which has been adopted by Chester in his valuable study of the classification of bacteria. As I have arranged these groups it results that in some cases there are placed together under one table the two genera which Migula has distinguished as *Bacillus* and *Bacterium*. According to Migula's classification a distinction between these two genera is based upon the formation of spores. The genus *Bacillus* produces spores, while the genus *Bacterium* does not produce spores. By the method of grouping which I have adopted, it has resulted that in groups IV. and VII. both of the genera *Bacillus* and *Bacterium* are included. This confusion, however, is not a serious one, since it involves only a few organisms. The grouping that has been adopted in the following pages is one which I have found to be the easiest to use in laboratory practice. The groups which I have recognized are as follows:

GROUP I. *Fluorescent bacteria.*

- II. *All red chromogenic forms.*
- III. *All orange chromogenic forms.*
- IV. *All lemon yellow chromogenic forms.*
- V. *All non-liquefying micrococci not included in II., III., and IV.*
- VI. *All liquefying micrococci not included in II., III., and IV.*
- VII. *All non-liquefying rods which are not chromogenic. These are mostly of the species Bacterium, but the table includes two of the genus Bacillus.*
- VIII. *All liquefying Bacteria without spores.*
- IX. *All liquefying Bacilli with spores no larger than the rods.*
- X. *All liquefying Bacilli with large spores causing the rods to be swollen at the time of sporulation.*

NAMING THE SPECIES.

In regard to the question of naming the species described, I have been somewhat at a loss as to the best method of procedure. Some of the species which are described are unquestionably entirely new and are very distinctly characterized. Others are very obscure in their diagnostic character, so much so that it has been difficult or impossible to give characters which very clearly define them. In these cases I have been

uncertain whether I was dealing with wholly new species or not. Some of the species described are very abundant and are found very frequently in dairy products, while others are rare, being found only once or twice. In my original laboratory notes each of these species has been entered by number. But it has seemed to me wisest in the following pages to apply names to such species as are clearly distinct and new. I have looked through the literature of systematic bacteria as carefully as possible, and wherever I could do so I have identified the species I have found with those described elsewhere. This, however, has not been possible in a majority of cases. It must be regarded as doubtful whether the identification of species found in milk with those found in water, soil, and air is accurate, and when the attempt is made to identify American species with those of Europe the uncertainty becomes very great. A few well marked species may be thus readily recognized, but for most species we must, in the present condition of bacteriology, recognize that any identifications of American dairy bacteria with bacteria from other sources and localities is very uncertain. For the most part the dairy bacteria must at present be considered by themselves. Where I have been able to class my species with those elsewhere described, the classification has been indicated by applying to the organism here described the name of the species with which it has been identified.

In regard to the rest of the species which cannot be identified with any described species, I have adopted the following plan. Wherever the species in question is an extremely common one or one that is very easily described and recognized from description, I have given it a specific name. In using these names I have in most cases added the word *lactis* for the purpose of indicating the fact that the organism in question is of a species found in milk. In the following pages, therefore, where a new name has been applied to a described organism this indicates either that it is a very abundant dairy organism or that it is one whose characters are so distinct as to indicate clearly that it differs from any other described species, and moreover so distinct that it can easily be recognized from the description. Where the organism in question, however, is found only rarely in dairy products and has characteristics so obscure as to make

it difficult to define it with accuracy, I have simply retained the original number by which this species has been entered in my laboratory notes. It is hoped that further study in future years may enable me to determine more accurately whether the species should be subsequently kept isolated and given specific names or whether they may eventually be merged into some of the other more common types.

METHOD OF TABULATION.

In the use of the tables the following methods have been adopted. At the top of the table in parallel columns are given certain characteristics which are indicated for each organism in the proper column by the sign + or —. The sign + indicates always that the species possesses the characteristic in question, the sign — that it does not possess the characteristic in question. In some places the sign ? has been inserted, which indicates that the characteristic in question is doubtful. For example, under the column headed "Diameter greater than 1μ " the ? means the diameter is practically 1μ . In the column referring to the reaction of milk, the ? sign indicates that the reaction is unchanged or is amphoteric. The other places where the ? sign is used explain themselves. In the use of this table the word bacillus merely refers to the fact that the organism is a rod rather than a coccus, and it does not mean that the organism is a bacillus in the sense of Migula's classification.

The tables when thus filled out give in a brief compass the chief diagnostic characters of the different species of bacteria. But these are not sufficient to give a complete description. There is therefore given in the pages following the table, under the proper numbers and names, such description of diagnostic characters as may be needed, in addition to those inserted in the tables, for the proper diagnosis of the species. By the use of the tables and these descriptions together each species is described as fully as possible from the data which are in my possession. These tables will be found extremely simple to use if one has a species of bacterium which he wishes to identify with those described. My method is as follows: I make a "trial slip" giving the characters included in the tables. Upon this slip I enter the characteristics of the species being studied with the + and — signs in spaces corresponding to

the columns in the tables. Then by moving the trial slip up and down the table it is possible to determine at a glance whether it agrees with any of the described species. If it is found that the characteristics agree practically with those of any species given in these tables, reference to the descriptions following will give further details and will make identification in most cases a simple matter. The whole comparison takes only a very few minutes and is a great saving of time over the old method of comparing long detailed descriptions.

THE CHIEF DAIRY SPECIES.

It is necessary to give here a brief statement concerning the bacteria which are to be regarded as the distinctive dairy organisms of this region. Although the number of species found in dairy products as seen by the following pages is large, the number of those which are found with very great uniformity in dairy products is small. Indeed, as the result of my experiments I have concluded that the dairy organisms of this region are chiefly of four species. Strictly speaking, it is probably more correct to say three groups of closely related bacteria rather than four single species. They are as follows:

The most abundant of our dairy organisms is No. 206, which is the *B. acidi lactici* Esten l. As already mentioned in a previous publication,* it is almost universally found in samples of milk or cream. This appears to be true not only of milk and cream in this region but of milk from a very wide territory. Samples of milk that have been sent us from a large number of States have in almost all cases shown the presence of this organism in abundance. In sour milk it is almost always present. In all samples of ripened cream which we have studied it has been found to be by far the most abundant species. In most samples of ripened cream this No. 206 forms over 75 per cent. of the bacteria present, and sometimes over 90 per cent.

The source of this organism in our milk has been a matter of some little interest, and has been studied by experiment in the last few months. Its almost universal presence in milk, together with the markedly anaerobic character, would seem to indicate that it probably comes from the milk ducts. This

* See Report of this Station for 1896.

conclusion has not been borne out by the direct studies of bacteria in the milk ducts. Our recent experiments seem rather to point to the conclusion that this organism comes from external contaminations. When we have collected samples from large numbers of cows, drawing the milk directly from the teats into sterilized vessels with little or no chance for contamination, it has been found that milk thus obtained only in very rare cases contains the organism No. 206. In the experiments, which now number over 200 and involve 75 well kept cows, there have been only five instances where such milk contained this organism, and the cows concerned in these five cases did not show the same result with a second test. The milk obtained directly from the cows in this way contained many species, commonly including liquefying bacteria, but not this common lactic species. When, however, the milk is drawn from the cow into sterilized vessels with a more widely open mouth, the organism in question has been found in most cases to be present, and becomes abundant in a few hours. This indicates that the *B. acidi lactici* should be regarded as an organism which comes from external contamination, and is not a normal inhabitant of the milk duct. On the other hand, up to the present time I have not succeeded in finding the organism in question present in any considerable numbers in gelatin plates which have been exposed to the air underneath the cow during the milking process. My present belief is that this organism is not a normal inhabitant of the milk duct, but commonly is derived from external sources, and is practically always present in the milk vessels into which the milk is drawn.

Next in abundance to the organism just described is No. 202. This may perhaps be a variety of the last, but it differs from it in being more markedly anaerobic, and in producing a colony in gelatin which is extremely minute and indeed invisible to the naked eye, whereas No. 206 produces a good sized colony. This is also very abundant in nearly all samples of sour milk or cream, although the numbers are much less than those of the last species. This organism I have not been able to find in the milk ducts any more than the last, and I regard it also as being derived from external contamination.

These two species undoubtedly belong together, not only from their morphological similarity and their general likeness

in growth upon various media, but also from their action on milk. They certainly represent a type of dairy organisms common everywhere. Many of the lactic organisms hitherto described by different bacteriologists clearly belong to this type, although slight differences in described characteristics perhaps indicate different varieties. This is true of the *Bacterium acidi lactici* of Günther and Thierfelder, *Bacterium lactis acidi* of Leichmann, *Bacillus XIX.* of Adametz, *Bacillus a.* of v. Freudenreich, *M. acidi laevolacidi* and *B. acidi laevolactici* of Leichmann and several of the types described by Storch. The pure culture used in cream ripening and put on the market by Hansen is a culture of one of this type of lactic organisms, and the same is true of the pure cultures of Witte and Barnekow. All of these organisms agree very closely in general characters and are doubtless closely related. In this region this type, represented by No. 206 and No. 202, is the most abundant milk bacterium, at least in milk obtained in the ordinary manner and allowed to stand for several hours before testing.

The next most important dairy species in this vicinity is No. 208, which I have regarded as identical with *B. lactis aerogenes*. This is found almost universally, although never in very great numbers. The organisms included under this number, however, have shown very wide variations, and it is quite possible that a number of distinct types are here included. At all events, it is quite certain that if all these forms are to be regarded as one species, several quite distinct varieties must be recognized among them. The distinctive characteristics of these species are, (1) the intense acid that the colonies produce in litmus gelatin; (2) the abundance of gas which is developed when they grow in milk sugar bouillon or in milk; (3) the uncertainty as to their power of curdling milk, this occurring commonly at high temperatures though not commonly at room temperatures; and (4) the distinctive odor of sour milk which is found in milk that has been curdled by means of them. *B. acidi lactici*, I. and II., Nos. 206 and 202, although they curdle milk with the production of lactic acid, do not give rise to the typical sour milk odor, and neither of them develop any trace of gas in the milk. I am therefore convinced that the ordinary souring of milk is produced in part by the action of this No. 208, and that typical sour milk, with its tendency to

fragmentation and its sour odor, is never developed unless some of the organisms included in my No. 208 species are present. Ordinary sour milk, according to my observations, is produced by these three organisms, and probably in the spontaneous souring of milk all three are present.

I am convinced that here also the various bacteria which I have included under No. 208 do not represent a single bacterium, but rather a group of allied varieties, and as a group represent a most important dairy organism. In looking through the literature upon dairy bacteria, it appears to me that many of the lactic organisms that have been described by different observers belong to this group. The original *B. acidi lactici* of Hueppe apparently belonged here. Here, too, probably must be placed *Bacterium lactis acidi*, Marpmann, *Bacillus lactis acidi*, Marpmann, *Bacillus acidi lactici*, I. and II., of Grotenfelt, No. 8 of Eickles, and doubtless several others. In the pure cultures used for cream ripening in European countries, known as the culture of Lorenz, the organisms appear to belong to this same type. The two forms recently isolated from Edam cheese by Leichmann and Bazarewski and called *Bacterium caci*, I. and II., also belong to this series. It is quite unlikely that these different organisms are the same, although their morphological and cultural characters in general accord. All of these facts indicate that in the species which I have at present called *Bacillus lactis aerogenes* there are grouped together a number of types with great similarity, but with at least different physiological characters.

The third type of bacteria which I have found so abundant as to call it a distinctive dairy bacterium of this region is my *M. lactis varians*, No. 113. This has been sufficiently described elsewhere, and as already mentioned is a very highly variable Micrococcus both as to chromogenic powers and power of liquefying gelatin. This species is common in fresh milk and probably exists in the milk ducts. It is commonly overgrown by the lactic organisms and is less common in old milk. It is by no means universally found and may be only a local species.

Several other species in my list are quite common in milk, but I think that these four must be regarded as the chief dairy bacteria of this region.

CLASSIFICATION OF DAIRY BACTERIA.

TABLE I.

[illegible]

Chromogenic type. (Orange.) Micrococci and Bacteria.

Chromogenic type. Lemon yellow.

GROUP VI.

GROUP VII.
Bacilli and Bacteria. Non-liquefying and non-
chromogenic.

Liquefying Bacilli, with large spores.

23. *

Large pores at the end of the rod

Two spots (?) in each row.

† Non-motile.

DETAILED DESCRIPTION OF BACTERIA.

GROUP I. FLUORESCENT BACTERIA.

Bacteria which produce a blue-green color either in agar or gelatin or bouillon or in all three.

No. 21. (Very common.) *B. fluorescens Schuykilliensis*. (?)
(Wright.)

Morphology; .8 μ by 2 μ . Chains.

Gelatin plate; a large liquefying colony, greenish and granular, later becoming surrounded by a clear pit. Later the granular center breaks up into a diffused mass.

Gelatin stab; a shallow pit, becoming deep and broad. Later a horizontal liquid layer is formed with a scum and a precipitate, and a clear, green liquid between. Growth is very slow.

Agar; a thin, white, not very opaque, moist growth. Agar green.

Potato; diffusely spreading, very thin, moist and brown.

Milk; sometimes curdles in 3 days at 20°, and at other times fails to curdle. Alkaline, and there is a slight digestion.

This bacterium appears to agree most closely with *B. fluorescens Schuykilliensis* (Wright), the differences being only within the range of variation.

No. 31. (Very common.) *B. fluorescens liquefaciens*.
(Flügge.)

Morphology; size, 1.5 μ by .9 μ , forming long chains.

Gelatin plate; small round colonies, with radiating marking under the surface. Surface colonies become surrounded by a clear, granular pit, the center gradually disseminating into the pit.

Gelatin stab; slowly liquefying in a rather deep funnel. The liquid is intensely green.

Agar; white, smooth, moist and glistening.

Potato; thick and brownish.

Bouillon; liquid becomes very green.

Milk; a soft, slimy curd is produced at 20°, which begins to digest at once into a yellowish green alkaline liquid.

This seems to be one of the varieties of *B. pyocyaneus* (Gessard), and closely related to *B. fluorescens liquefaciens*. It is a very slow liquefier.

The many cultures of No. 21 and No. 31 which have been isolated and studied show considerable variation, and possibly several varieties have been here included which might be properly separated. But since they seem to belong to two types, I have recognized only the two above described.

Both of these species have been found many times in milk from all the localities studied, and while these organisms cannot be regarded as distinctively characteristic of milk or cream, they are so commonly found that their presence is not unusual. Whether they indicate, as has been sometimes assumed, that the milk has been adulterated or contaminated with water is a question that I do not feel that I have data for determining.

No. 128.

No. 128 is only slightly different from No. 21 and No. 31. The chief differences are the following: Its size is considerably smaller, being only .5 μ by .7 μ .

whereas the other two, have a diameter of $.8\mu$ and a length of 1.5μ . In gelatin stab culture No. 128 produces a very shallow pit, whereas No. 21 and No. 31 produce a deeper pit; and, moreover, the liquefied gelatin is not rendered green, or only slightly so, by No. 128. The effect of this organism upon the milk is also very slight. While the milk is curdled in two or three days, there is little or no digestion of the casein; whereas, as shown by the tables, the casein is readily digested by Nos. 21 and 31.

That Nos. 21 and 31 are varieties of the same species seems to me almost certain. No. 128 is probably a different variety of the same species, and possibly is *B. fluorescens minutissimus*. (Unna.)

No. 5. *B. viscosus*. (Frankland.)

Morphology; size, 1μ by 2μ , but variable. Showing bi-polar staining. No threads are produced, but a capsule is present. No true spores, but the double stain gives the appearance of spores.

Gelatin plate; a small white colony is produced, which sinks into a pit and rapidly liquefies the gelatin. The pit has a granular center and a clear rim, and grows into a uniformly granular colony with a radiating rim. Only a slight tinge of green.

Gelatin stab; a narrow funnel is produced, with a thick tenacious scum on the surface, with a tendency to crack and wrinkle. The liquid becomes green and later yellow and slimy.

Agar; upon agar there is produced a thin, rough, white, dry skin, which is marvelously sticky, almost like glue. The agar becomes slightly green.

Potato; abundant, brownish yellow growth.

Milk; the milk is curdled at the room temperature in two days into a soft, slimy mass, with no whey, the reaction being alkaline. This becomes rapidly digested into a lemon yellow liquid; after the casein is all dissolved, the whole liquid is yellow and slimy. The odor is sickish, and the mass is very slimy. At 35° there is no digestion. It is further to be noted that after cultivation of about four months in the laboratory, this organism ceased to have the power of coagulating milk, although it continued to digest the casein at room temperature. The production of the lemon color also ceased.

The most distinctive characters of this organism are its *bi-polar staining and its production of a slime*. Experiments were performed with this specie, showing that it produced a soluble enzyme. The organism was cultivated in milk for some time, and then filtered through porcelain. The filtrate, which contained no bacteria, was found to have the power of coagulating milk in an hour's time at room temperature. From such filtrate an enzyme was isolated, as described in a previous publication.*

This organism appears similar to *B. viscosus*. (Frankland.)

Nos. 82 and 90. *B. fluorescens non-liquefaciens*.

Nos. 82 and 90 are without much doubt the same organism, and are probably identical with *Bacillus fluorescens non-liquefaciens* of Flüggé. Slight differences led me to recognize two varieties, which appear in the descriptions. The organisms are not very common.

* Cent. f. Bact. u. Par., XII., 1892.

Morphology; a bacillus .6 μ by 1.2 μ to 2 μ . No. 82 forms chains, while they were not found in No. 90.

Gelatin plate; the colony of No. 82 is at first round and opaque, but white. When reaching the size 1 mm. it becomes surrounded by a green halo. Later it becomes rough, irregular in shape, but still surrounded by the green halo. The colony of No. 90 spreads over the surface in a bluish white, clear colony, which frequently shows a darker granular center. No green halo appears.

Agar; a very thin, hardly visible growth is produced, which spreads over the surface, and the agar is turned green.

Bouillon; there is formed a thin scum with a very slight cloudiness, and subsequently a sediment appears. After several weeks the liquid is quite cloudy and a sediment is noticeable, which is greenish in the case of No. 82, and brownish in the case of No. 90.

Milk; this organism produces no noticeable effect upon milk at any temperature.

Both of these cultures were inoculated into pasteurized cream, and after the cream was allowed to ripen 24 hours it was churned. The butter produced had a moderately good flavor, though not strong, and hardly different from butter made from pasteurized cream without inoculation.

These six varieties which, as indicated, probably belong to four species, include all of the fluorescent forms which have been found in the dairy products in this vicinity.

GROUP II. CHROMOGENIC TYPE. (RED.)

No. 209. *Bacillus prodigiosus*.

Bacillus prodigiosus has been found a few times in milk in this vicinity.

No. 62. *Micrococcus rubidus lactis*. (n. sp.)

This species has been found only once, but it appears to be different from any previously described bacterium. It is a coccus form, *non-motile*, and fails to render milk red or to curdle it. In other characters it resembles *B. prodigiosus*.

Morphology; a coccus about 1 μ , enveloped in a non-staining capsule.

Gelatin plate; rapidly liquefying colonies, commonly with red pigment, although many colonies fail to produce the pigment.

Gelatin stab; a narrow funnel, soon widening and depositing red pigment. The whole gelatin liquefies and becomes red.

Agar and potato; a blood red, thick, luxuriant growth. Pigment not produced at 35°. Pigment most profuse at 23°.

Milk; no curdling, although the milk becomes thickened slightly, with red margin. No change in reaction. In old cultures a mass of casein is seen floating in a clear liquid.

No. 42. *Micrococcus rosaceus lactis*. (n. sp.)

This specimen was obtained originally from Uruguay, but there was later isolated from milk in Middletown a culture that agreed with it in all essential respects.

Morphology; a micrococcus, .8 μ in diameter, grouped in fours.

Gelatin plate; colonies 1 mm. in diameter, of a light pink color. Under a low power they show a nucleus with a lighter zone. Upon the surface the edges are thin and the center raised.

Gelatin stab; there is a slight needle growth and a slight surface growth, which is pink.

Agar and potato; a moist, thick, not widely spreading, pink layer.

Bouillon; after a few days a pinkish sediment makes its appearance. The liquid is cloudy, with no scum.

Milk; no curdling takes place. Reaction becoming slightly alkaline, with no digestion. After two weeks a very slight pinkish tinge is noticed in the milk, which becomes quite decided in a month. Later a pink scum forms, and the milk becomes somewhat slimy.

The distinctive characteristic of this species is the *pink color* produced, which shows in the gelatin colony, in the gelatin stab growth, in the growth on agar and potato, and in the sediment in the bouillon.

No. 115. (Somewhat common.) *Bacillus ruber lactis*. (n. sp.)

Morphology; a rod .9 μ by 2 μ to 4 μ in length, occasionally forming long bent chains.

Gelatin plate; a white opaque bead, .7 mm. in diameter, formed on the surface of the gelatin, coarsely granular and broken around the edge. Gelatin liquefies rapidly, with a central mass and an outer granular zone, which is broken and lobed at first. Later the outer zone becomes clear as it expands, and the inner mass is broken into fragments.

Gelatin stab; a shallow funnel formed, with a thin liquid layer of gelatin over the whole surface of the tube, which gradually deepens. A dense sediment appears in a clear liquefied gelatin, but no scum.

Agar; a thick, coarsely folded growth, which may be at first yellow, but later *pink*.

Potato; smooth, thick, glistening growth, with a decidedly *pink* or *salmon* tinge. A very striking characteristic.

Milk; curdled in 4 days at body temperature. The curd is soft and alkaline, and soon digests into a colorless liquid. At 20° the milk does not curdle, but digestion occurs. When used for ripening cream it produces a good flavored butter, with a pleasant aroma, though neither taste nor smell are those of typical butter.

No. 151. (Rare.)

Morphology; Bacillus .6 μ by .7 μ to 1.2 μ , blunt ends.

Gelatin plate; surface colony round, semi-transparent, liquefying gelatin slowly, with an *orange pink* color.

Gelatin stab; slight needle growth, with slow liquefaction. A slightly cloudy liquid is produced, with a thin scum.

Agar; moist, salmon pink growth.

Potato; a moist, light orange growth, which later becomes deeper and shows various shades, from *orange* to a *brilliant red*.

Milk; no effect for 10 days, except a slight pink scum around edge and a pink tinge to milk. Then it curdles into a soft curd, which later digests into an orange colored liquid. Is alkaline.

The two following groups, III. and IV., are separated from each other by the color of a pigment which they produce, the one giving a *lemon* yellow and the other an *orange* yellow pigment. It has been found by experience that brownish colors, especially upon potato, have no significance, but that lemon and orange yellow colors are quite distinct from each other, and may be used to characterize different groups. Occasionally forms may be found in which it is a little difficult to determine to which of these two groups they belong. For convenience in classification some of these forms have been included under both groups, but as a rule the yellow and orange are sharply separated.

GROUP III. CHROMOGENIC TYPE. (ORANGE.)

No. 199. *Sarcina flava*.

This species has been occasionally found in milk.

No. 188. *M. aureus lactis*. (n. sp.)

Morphology; a coccus, size, $.8\mu$, in pairs or in clumps.

Gelatin plate; a round, opaque colony, surrounded by a halo which is uniformly granular, somewhat indented and cracked. This increases to a large, uniformly granular liquid zone, which spreads in all directions. The liquefaction at first is chiefly below the surface.

Gelatin stab; a shallow pit is produced, which deepens into a horizontal layer with a yellow sediment and slightly cloudy liquid. Liquefaction becomes complete.

Agar and potato; an abundant, moist, glistening Naples yellow growth.

Milk; after three weeks becomes curdled and rendered alkaline. Later is partially digested into a transparent liquid, with considerable undigested sediment.

No. 103. (Quite common.) *B. aureus minutissimus*. (n. sp.)

Morphology; a bacillus, size, $.4\mu$ by 1.6μ . Three or four may be united together, and in bouillon, long tangled threads.

Gelatin plate; surface colony at first thin, irregular, branching and creeping. The deeper colonies are *burr like*, with a yellow center and radiating processes. After two days a liquefying pit is formed, with a yellow center and *irregular processes* extending into the gelatin. The whole is quite characteristic.

Gelatin stab; a deep, narrow funnel, with a brilliant yellow sediment and scum, and a somewhat cloudy liquid.

Agar; an orange yellow growth, spreading over the whole surface.

Potato; a dark orange growth of a very deep color and striking appearance.

Bouillon; a slight scum on a uniformly cloudy liquid, and a yellow sediment collects after some weeks.

Milk; at 20° becomes somewhat pasty and dark colored. Slightly slimy, and is alkaline in reaction. Butter made from cream ripened with this organism develops an aroma of decay which is unpleasant. No very decided flavor.

Nos. 113 and 104. *Micrococcus varians lactis*. (n. sp.)

This is one of the most common of our dairy species, being found very commonly in milk and cream. It is frequently found in plates made by collecting dust that falls from the body of the cow during milking. It is a very widely variable species.* Its power of producing pigment varies from a deep orange to a pure white. It commonly liquefies gelatin rapidly, but some cultures have been found with this power only slightly developed and some in which it is wholly absent. In the table I have included two of the extreme types, and the variations are mentioned below.

Morphology; a coccus form, slightly variably in size, but about 1μ in diameter. It never forms chains, and stains easily.

Motility; none.

Temperature; grows readily at ordinary temperature. Grows rapidly at a temperature of 38° , but with less color.

Mica plate; grows under the mica plate, but not much in the middle. Evidently an aerobe with slight anaerobic powers.

Gelatin plate; colonies at first forming a whitish or yellowish bead on the surface, which sinks into a slight pit with an irregular edge. The pit broadens, liquefying the gelatin rapidly, and the colony breaks up into irregular yellow masses. The pit is sometimes very deep, and contains the irregular floating masses of bacteria. The general character of the colony is very characteristic, and can be readily distinguished at a glance from other liquefying colonies. The non-liquefying variety, No. 104, simply forms a yellow colony, not characteristic.

Gelatin stab; a broad, shallow funnel is produced, with a broken yellow scum and a yellow flaky sediment. Sometimes there is liquefaction along the needle track, and sometimes not. The liquefaction is rapid, and in a few days the gelatin is completely liquefied. No. 104 forms a shallow, dry pit, with a dense yellow surface growth.

Agar; a very characteristic, dry, rough, yellow growth. The color is slightly orange, though not very deep. From this the color varies to a pure white.

Potato; a dry, granular, orange yellow growth, abundant and characteristic. Color varies to a white, and sometimes the growth is moist rather than dry.

Bouillon; in two days a slight cloudiness is produced. In six days the liquid is very cloudy, but with no sediment. In four weeks very cloudy, with a yellow sediment.

Milk; curdles at 36° in three days into a soft curd, with an amphoteric reaction. At 20° it curdles in the same way in ten days. The curd is not subsequently digested, or only very slightly. When used for ripening cream in butter making, it produced very little flavor or aroma.

No. 159. (Rare.)

Morphology; a bacillus, size, $.7\mu$ by $.9\mu$.

Gelatin plate; characteristic. A mounded, yellowish, spreading colony, which becomes 1 centimeter in diameter, thin and almost invisible on the edge. Is irregular shaped and very yellow.

* The variations of this species have been previously described in the Cent. f. Bact. II. Par., II., V., p. 665.

Gelatin stab; a good needle growth, with a thick yellow orange surface growth.

Agar and potato; thick, moist, glistening yellow, of a very deep orange shade.

Milk; no effect produced on milk.

Nos. 162 and 141.

See the same numbers under Group III. They are listed here also, since the pigment sometimes approaches an orange color rather than a lemon yellow.

No. 169. (Rare.)

Morphology; a bacillus, size, $.5\mu$ by 1μ . Has an irregular stain, with light spots in the middle that are not true spores. On potato they grow to a length of 4μ , with square ends, still showing irregular stain.

Gelatin plate; deep colonies are round and yellow, with a dark center and a yellow rim. Surface colonies are about .5 mm. in diameter. They are yellow and transparent, the transparent colony being the most distinctive character.

Gelatin stab; an abundant needle growth, with a thin, widely spreading, transparent surface.

Agar; an orange yellow, transparent, moist growth.

Potato; A widely spreading, moist, orange growth, sometimes very deep in color and almost brown.

Bouillon; orange flakes appear on the surface, and later a yellowish sediment.

Milk; is not curdled, but an orange scum and an orange sediment are produced, and the milk rendered alkaline.

No. 170. *B. aureus acidi*. (n. sp.)

Morphology; a bacillus, size, $.6\mu$ by $.7\mu$. Occasionally somewhat longer.

Gelatin plate; colonies under the surface round, yellowish and slightly opaque. Surface colonies rather transparent, spreading, slightly irregular, 1.5 mm. in diameter, and of an orange color.

Gelatin stab; a deep, dry pit is produced, with a dry orange yellow skin lining the pit.

Agar and potato; both show an orange yellow growth.

Milk; is curdled in from two to four weeks into a moderately hard curd, which is acid, showing no whey and having no digestion.

No. 205. *B. aureus lactis* I. (n. sp.)

Morphology; a bacillus, size, $.6\mu$ by 1μ , with rounded ends. No chains, though three or four may adhere together.

Gelatin plate; under the surface a yellowish orange, slightly irregular colony. On the surface an orange bead, .5 mm. in diameter, which is in the middle of a slightly depressed ring, but no liquefaction of gelatin occurs.

Gelatin stab; a good needle growth and a slight orange surface growth appears.

Agar; a moist, thick, smooth ground glass growth, orange yellow in color.

Potato; a dry, or moist, orange growth.

Bouillon; an orange yellow, tenacious scum appears with a clear liquid.

Milk; no effect produced, except an orange scum and an alkaline reaction.

No. 100. *B. aureus lactis* II. (n. sp.)

Morphology; a bacillus, size, $.5\mu$ by $.7\mu$. No chains, though two or three adhere together.

Gelatin plate; a bead with a smooth edge and a dark center is produced, 1.5 mm. in diameter, which, after a few days, becomes decidedly yellow.

Gelatin stab; a slight needle growth, with an irregular, opaque, white surface growth, not very thick.

Agar; smooth, whitish growth, which, after a few days, acquires a lemon color, and later a Naples yellow color.

Potato; an abundant growth, which is at first white, or slightly yellow and quite thick, later becoming decidedly yellow.

Bouillon; a thick, *tenacious scum* is produced, which sinks in the form of flakes, and produces a sediment.

Milk; no effect produced on milk, except that a slight slimy scum sometimes appears. Butter made from cream ripened with this organism has a prominent flavor, which is not normal and unpleasant. There is a slight and tolerably pleasant aroma. The butter on the whole is of a good quality. Nos. 205 and 100 are perhaps the same.

No. 137.

Morphology; a bacillus, size, $.6\mu$ by 1.2μ , or occasionally larger, with round ends.

Gelatin plate; the deep colonies are round and slightly granular. On the surface they spread into a thin, transparent colony, which later becomes thicker and brown and yellowish. It may occasionally form a thick, yellowish bead.

Gelatin stab; a moderate needle growth, with a yellowish irregular surface growth, with a slightly raised edge.

Agar; a not very abundant dull yellow growth.

Potato; spreads over the surface of a thin, decidedly yellow growth.

Milk; commonly curdled at room temperature in about two weeks, though sometimes becomes simply slightly lumpy. At body temperature it curdles completely, though the curd is rather soft. The action is amphoteric, and there is no digestion. No effect on butter.

No. 78. (See Group V.)

GROUP IV. CHROMOGENIC TYPE. (LEMON YELLOW.)

Nos. 48 and 116. *B. lactis erythrogenes*. Varieties I. and II.

These two cultures I regard as varieties of *B. lactis erythrogenes*, although one is a typical bacterium and the other a coccus. Variety I. appears to agree with *B. lactis erythrogenes* of Hueppe. They have each been found several times and differ from each other in a few constant characters. But since the few differences remained constant with cultivation, I have found it convenient to separate them as Varieties I. and II. Variety I. liquefies gelatin very slowly, or not at all, while Variety II. liquefies rapidly. Variety I., moreover, does not grow on potato, and turns milk red; while Variety II. grows on potato, forming an abundant yellow growth, but it does not turn milk red. The characters of Variety II. are as follows:

No. 116. *B. lactis erythrogenes*. Variety II.

Morphology; a large coccus, $.8\mu$ in diameter.

Gelatin plate; the colony is at first a bead, or flat and brownish under the microscope. Grows into a flat colony, 1.5 mm. in diameter, in four days, and then sinks into a shallow pit.

Gelatin stab; an abundant needle growth, white, with a flat, white surface growth sinking into a shallow pit, the bacteria mass forming a dense scum on the surface of the liquefying gelatin. Liquefaction occurs slowly and horizontally.

Agar; a thick, white, opaque growth, giving to the agar a *pinkish tinge*, which later becomes somewhat red. The growth on the surface becomes yellow.

Potato; an abundant yellow, moist, opaque skin.

Milk; is rendered slightly alkaline, but not curdled. After four weeks it is digested into a watery or semi transparent liquid, yellowish in color, with a peculiar smell. It produces no effect upon butter when used for cream ripening.

No. 174. (Uncommon.)

Morphology; a bacillus, size, $.9\mu$ by 1.5μ , with rounded ends. No chains, though three or four may hang together.

Gelatin plate; the deep colonies are oval, dark, opaque, and the surface colonies are at first white and about half a millimeter in diameter, thin, sinking into a pit, with a large, yellowish nucleus.

Gelatin stab; a moderately shallow funnel is produced, gradually liquefying, with a cloudy liquid and abundant sediment, but no scum.

Agar; slightly lemon yellow growth on the surface, and the agar acquires a *pink tinge*.

Potato; white or yellow growth, which later becomes quite abundant and lemon yellow.

Milk; curdles at body temperature in three days into a soft, alkaline curd. Digests into a cloudy, colorless mass, which sometimes may be reddish yellow or amber colored, and with a slimy scum.

This organism is very similar to No. 116, above described, and is perhaps identical with it. The pink fluorescence is very slight, and the lemon yellow color more noticeable, and this has led me to separate the two.

No. 201, *Sarcina lutea*, and No. 199, *Sarcina flava*, have both been found occasionally in dairy products.

No. 117. (Rare.). *Micrococcus citreus lactis*. (n. sp.)

Morphology; a coccus, $.9\mu$ in diameter.

Gelatin plate; a smooth, opaque surface colony, 1 mm. in diameter, which in about five days becomes 2.5 mm. in diameter; very *thin and flat and decidedly yellow*. Gelatin ordinarily becomes dry before liquefaction begins.

Gelatin stab; needle growth abundant. A flat, depressed, yellow surface growth, which is sunken in the middle and slowly spreads over the surface of the gelatin. After about two weeks a slow liquefaction begins, with a floating scum on the surface of the liquid. (One variety of this species was found which liquefied the gelatin more rapidly and produced a narrow funnel.)

Agar; abundant moist growth of a brilliant yellow.

Potato; a rather dry but abundant lemon yellow growth.

Milk; rendered strongly alkaline, but no further change produced. No noticeable effect produced upon butter when used in cream ripening.

No. 107. (See Group VI.)

I regard this as a variety of No. 117. It grows, however, at 35°, and under a mica plate, and liquefies more slowly.

No. 91. (Rare.) *B. citreus acidi*. (n. sp.)

Morphology; a bacillus, size, .5 μ by .8 μ , with no chains.

Gelatin plate; large, white, opaque colony, becoming 2 mm. in diameter, and later turning yellow.

Gelatin stab; a good needle growth, with a spreading surface, slightly raised on the edge and depressed in the centre. This becomes lemon yellow and spreads slowly over the surface.

Agar; an abundant, spreading, lemon yellow surface growth.

Potato; a thick, white and slightly transparent growth, the center of which soon becomes yellow, and later the whole turns to a lemon yellow.

Bouillon; a slight scum is formed, which sinks in the form of flakes and produces an abundant sediment.

Milk; curdled at ordinary temperatures in 6 to 9 days with a clear, hard curd and a deep yellow layer of milk on top. Is acid and has a decidedly sour odor, with no digestion. Cream is also curdled and filled with gas bubbles. It is acid, and has a pleasantly sour odor, with no separation of whey. Used for cream ripening, it produces a good butter flavor, though slight, but no aroma.

No. 72. (Rare.)

Morphology; a bacillus, size, .8 μ by 1.4 μ . Produces very short chains with spores. In old cultures long threads may be developed.

Gelatin plate; produces a perfectly transparent surface colony, with an irregular edge, half a millimeter in diameter.

Gelatin stab; a moderate needle growth and a dry, white, moderately abundant surface growth.

Agar; opaque, white and shining at first and abundant. Later becomes a lemon yellow.

Potato; thick, smooth growth, which may be white where moist, but soon becomes lemon yellow.

Milk; curdles at 36° in two days, but not at room temperature. Is acid. No effect is produced on cream, except an acidity and sour smell and taste. Butter made from such cream has a sour, unpleasant taste. When fully ripened, has an unpleasant aroma, and is decidedly poor.

No. 105. (Rare.)

Morphology; a short rod, size, .5 μ by .9 μ .

Gelatin plate; colonies under the surface are rounded or oval, with a dark center and a lighter outer zone, which is sometimes lobed or striated. On the surface large, moderately opaque beads are formed.

Gelatin stab; slight needle growth, but a moderately thick, white surface growth, which later becomes dry, with a slight yellow tinge.

Agar; the agar develops on the surface a very decidedly lemon yellow growth, which is thick and abundant. The agar may, at the same time, be turned green, but not universally so.

Potato; growth thin and moist, but lemon yellow.

Bouillon; slightly cloudy, and with a slight tinge of green near the surface.

Milk; no effect upon milk at any temperature. Butter made from cream ripened with this species has no special flavor or aroma.

No. 149. (Common.) *B. citreus lactis* I. (n. sp.)

Morphology; a rod, size, 1μ by $.7\mu$, with rounded ends. On potato and agar the rods are connected with a slimy capsule.

Gelatin plate; a minute colony, 1 mm., or a little larger. On the surface there is frequently produced a raised bead, with a central dot. The color is lemon yellow, brilliant, and even under the microscope the colonies appear brilliant lemon yellow, smooth and clear.

Gelatin stab; needle growth moderate to the bottom of the tube. A rough surface growth, rather thick, but not opaque.

Agar; a thin, moist, transparent lemon yellow growth.

Potato; a moderately thick, brilliant lemon yellow growth.

Milk; no effect is produced on milk or upon cream.

While quite similar to No. 105, this species differs in its gelatin colony and its intense lemon yellow pigment. It is quite common in milk.

No. 161. *B. citreus lactis* II. (n. sp.)

This organism agrees with the last described in all respects, except two. First, it liquefies gelatin slowly, producing a dense yellow liquid in the gelatin stab. Second, associated with this characteristic it is found that it curdles milk, producing a weak alkaline curd at ordinary room temperature.

These two organisms are apparently the same species, differing in the power of liquefying gelatin, and consequently curdling milk.

Both No. 149 and No. 161 have been found quite commonly in the milk in this vicinity, and in one case both of these types have been isolated from a single colony.

No. 187. (Rare.)

Morphology; a rod, size, $.8\mu$ by 1μ to 1.2μ . No chains, spores nor capsules.

Gelatin plate; a round, semi-opaque, slightly yellow colony produced, with no characteristic features.

Gelatin stab; a moderate needle growth, with a slightly raised surface growth. Faint yellow, but not characteristic.

Agar; a moist, smooth, glistening, lemon yellow growth.

Potato; an abundant, moist, glistening, lemon yellow growth.

Milk; no effect upon milk or cream.

This organism has no characteristic features, except the lemon yellow color which is produced on gelatin, agar and potato. I separate it from No. 149 because of the very moderate pigment it produces.

No. 141. (Rather common.)

Morphology; a bacillus, size, $.6\mu$ by 1μ to 2μ .

Gelatin plate; the deeper colonies are round, brown and opaque. Surface colony is more transparent, whitish and finely and uniformly granular.

Gelatin stab; a moderate needle growth, with a dry, white, spreading surface growth. Moderately thick. Somewhat irregular edges.

Agar; a thick, moist growth, which is at first white, with a tinge of yellow, later becoming lemon yellow.

Potato; coarse or fine folded skin, with a yellow color.

Milk; no effect produced on milk or cream.

No. 162.

This agrees with No. 141 in all respects, except that the growth on potato is very scanty, and there is scarcely any growth in bouillon. The two probably are the same.

No. 191. (Rare.) *B. citreus arborescens*. (n. sp.)

Morphology; a rod, size, $.8\mu$ by 4μ . Two or three may adhere together, but no long chains. Is joined by a capsule that does not stain.

Gelatin plate; a widely spreading colony, with *fine radiating rods* growing from the center, and some growing over the whole plate, with fiber permeating the gelatin in every direction. *These fibers have frequent knobs*. The fibers from two colonies will extend over a whole plate in three days. To the naked eye they look like a mould. This growth is very characteristic.

Gelatin stab; needle growth is slight, but a thick *ground glass surface* growth is produced.

Agar; white, moist and irregular, spreading in streaks over the surface.

Potato; dry and thin, but lemon yellow in color.

Milk; no effect, except a slight transparency and an alkaline reaction.

GROUP V. NON-LIQUEFYING COCCI, NOT CHROMOGENIC.

Division A.

Organisms which curdle milk with an acid reaction.

No. 60. (Very common.) *M. acidilactici* I. (Marpmann.)

Morphology; a coccus, $.6\mu$ in diameter, growing in masses.

Gelatin plate; forms rounded beads, finely granular, but with a smooth edge, and not characteristic.

Gelatin stab; moderate needle growth, rough and beaded. The surface is rough and irregular, moderately thick.

Agar; an opaque, white growth, which may grow down into the agar from the needle track, later becoming Naples yellow.

Potato; white, somewhat thick and spreading, and later becoming yellow.

Milk; at 20° is *rendered acid*, but not curdled, though such milk will curdle when boiled. At 35° is curdled into a hard curd which is *acid*. Cream is slightly thickened, rendered acid and sour. Butter made from the same has a decidedly good flavor, but practically no aroma.

No. 78. *M. acidi lactici* II.

This organism agrees with No. 60, except that it occasionally produces yellow pigment, and does not so readily grow without oxygen. The two are probably the same, and they are very similar to the following.

No. 58. *M. acidi lactici* III.

Morphology; diameter, $.8\mu$.

Gelatin plate; a colony, at first yellowish, then raised into a white bead. Not characteristic.

Gelatin stab and agar; not characteristic.

Potato; an extremely abundant, thick, shining growth. It may sometimes be 2 mm. to 3 mm. in thickness, of a flesh color, and is especially characteristic.

Milk; is rendered strongly acid, but does not curdle at 20° , though it curdles at 35° . Cream acquires a rather strong, sharp, penetrating, pleasant odor.

These three organisms appear to me to be similar to *B. acidi lactici* of Marpmann.

No. 168. (Rare.)

Morphology; coccus, $.9\mu$, and diplococci.

Gelatin plate; the deep colonies are round and opaque. On the surface they grow into a snow white bead, extremely opaque, which grows to the size of 2 mm., and then sinks into a pit, which sometimes liquefies and sometimes does not.

Gelatin stab; a beaded needle growth. On the surface there is formed a white skin, which sinks into a pit, commonly dry, though sometimes with a slight liquid.

Agar; an abundant snow white growth.

Potato; a hardly visible thin white streak.

Milk; is rendered acid and curdles when heated, but does not curdle normally.

This organism may be identical with No. 147 (Group VI.). As seen from the above description, it is probably a liquefier whose power of liquefaction is sometimes completely lost. In other respects it agrees very closely with No. 147.

No. 130. (Rare.) *M. viscosus lactis*. (n. sp.)

Morphology; a coccus, $.9\mu$ in diameter.

Gelatin plate; surface colonies are smooth and shining white; colonies $\frac{1}{2}$ mm. in diameter, and not opaque.

Gelatin stab; slight needle growth. An abundant shining white surface growth, raised into a mound.

Agar and potato; not characteristic.

Milk; becomes acid, but does not curdle. It soon becomes *extraordinarily slimy*, and can be drawn out into long threads. The sliminess does not affect the churning of the cream, and produces no flavor or aroma in the butter.

This coccus does not appear to be like any of the previously described slimy milk bacteria, and I have therefore regarded it as new, and given it the name, *M. viscosus lactis*.

Division B.

Organisms which do not curdle milk nor render it acid.

The first three agree in producing chains (*Streptococci*), and differ only slightly from each other. They may perhaps be the same.

No. 70. (Common.)

Morphology; .6 μ in diameter, forming chains in bouillon.

Gelatin plate; a surface colony, 1 mm. in diameter, rather opaque, with an irregular border, or sometimes a thick, raised bead.

Gelatin stab and agar; not characteristic.

Potato; cream white or yellowish white. Abundant and somewhat transparent, moist and *slimy*. Used for ripening cream, it produces a good flavored butter, without any sour taste.

No. 75. (Common.)

Morphology; diameter, .6 μ ; in bouillon growing into chains, with a capsule.

Gelatin plate; smooth, thick and transparent, 2 mm. in diameter, with occasional tendency toward roughness, and warts on the surface.

Gelatin stab; a slight needle growth, tapering rapidly below the surface. The surface is rather thick on the edge, with a smooth center. Is dry and transparent.

Agar; is yellowish white, quite thin and *dry*, and not widely spreading.

Potato; a dirty white or *snow white growth*. Used for cream ripening, it produces butter without taste or flavor.

No. 186. (Common.)

Morphology; diameter, .8 μ to .9 μ , occasionally forming short chains of 6 to 20 elements, especially in bouillon.

Gelatin plate; a round, opaque, slight yellow colony, spreading into a white, slightly raised colony, finely granular, .5 mm. in diameter.

Gelatin stab; a typical *nail colony*, with a round, smooth head, quite thick, at first white, but later showing a tinge of yellow.

Agar and potato; not characteristic.

No. 80. (Rare.)

Morphology; diameter, 1 μ to 1.2 μ . Slightly longer than broad. Forms chains of 6 or 8, which resemble rods, but which break up into cocci in old colonies.

Gelatin plate; a slightly granular, white rather transparent colony, 1 mm. in diameter. Not characteristic.

Gelatin stab; needle growth slight; thin, smooth, semi-transparent surface growth.

Agar and potato; not characteristic.

Milk; is rendered somewhat *slimy* and alkaline, but otherwise unchanged. Produces butter without flavor or aroma.

No. 118. (Rare.) *M. giganteus lactis*. (n. sp.)

Morphology; extremely large coccus, 1.5μ in diameter.

Gelatin plate; produces an opaque, white bead. Not characteristic.

Gelatin stab; an abundant needle growth, but no surface growth.

Agar and potato; no visible growth, and the same is true of bouillon.

Milk; produces no effect upon milk.

The essential characteristics of this organism are its *very great size* and its markedly *anaerobic characters*. One type, probably the same, produces a very slight *acid* reaction in the milk after four weeks growth, sufficient to curdle the milk when heated.

No. 47. (Uncommon.)

Morphology; $.6\mu$ in diameter.

Gelatin plate; a round, raised, white bead, 1 mm. in diameter.

Gelatin stab; an abundant needle growth, with a thick, moist but not widely spreading surface.

Potato and agar; *snow white*, moist growth.

No. 121. (Somewhat common.) *M. arborescens lactis*. (n. sp.)

Morphology; diameter, $.7\mu$. No chains.

Gelatin plate; deep colonies are irregular, granular, with a broken *fuzzy edge*. On the surface they are more regular, and become 1 mm. in diameter. There is great variation in density and in the amount of irregularity.

Gelatin stab; needle growth with *radiating fibers*. The surface growth is white, not thick, and spreads over the surface.

Agar and potato; not characteristic.

Bouillon; a very tough, *tenacious scum* is formed, which does not sink in the liquid.

No. 85. (Somewhat common.)

Morphology; $.6\mu$ in diameter.

Gelatin plate; a transparent bead, with a darker center and a scalloped border, 1 mm. in diameter. This bead is rather transparent, and spreads slightly.

Gelatin stab and agar; not characteristic.

Potato; thick and whitish, or *yellowish*, or sometimes *creamy white*. In cream the organism produces a pleasant odor, but butter made from it has no flavor or aroma.

GROUP VI. MICROCOCCI. LIQUEFYING, BUT NON-CHROMOGENIC.

No. 167. (Somewhat common.) *M. citreus lactis*. (n. sp.)

Morphology; a coccus, 1μ in diameter, in groups of 4, or irregular masses.

Gelatin plate; produces an opaque bead in a slight depression, which increases to 1 mm. in diameter, then sinks in a pit, in which the bead remains for some time in the center as a raised mound. The pit may be granular and either circular or lobed.

Gelatin stab; needle growth abundant, with not much surface at first. Later a deep, dry, narrow pit is formed, with a dense, white growth covering its walls. Still later the gelatin liquefies, and a yellowish sediment is produced.

Agar; opaque, abundant growth, with a tinge of yellow.

Potato; rather dry, whitish or with a yellowish tinge.

Bouillon; becomes uniformly cloudy, with no scum and with a *slimy* sediment. Another culture of what I regard as the same species produces a liquid pit instead of a dry pit, as here described.

Milk; culture does not curdle milk. This is the only one of the liquefying cocci found that fails to curdle milk.

I regard this as identical with No. 117 of Group IV.

No. 87. (Rare.)

Morphology; a minute cocci, $.3\mu$ to $.4\mu$ in diameter.

Gelatin plate; a small, granular colony, surrounded by a clear, liquefying ring. Later the nucleus breaks up, diffusing through the liquid, sometimes regular, sometimes irregular.

Gelatin stab; growth is slow. A narrow pit is formed, with an air bubble at the surface. Liquefaction complete in six weeks. Liquid is cloudy, with a heavy sediment.

Agar and potato; dirty white growth. Not characteristic.

Milk; is curdled, with weak alkaline reaction, in two days, at room temperature. At 36° in three days. A digestion follows, which is never complete.

Chiefly characterized by its *minute size*. Found only once.

No. 109. (Rare.)

Morphology; a coccus, 1μ in diameter.

Gelatin plate; a round, smooth surface colony, moderately transparent. Grows to the size of 1.5 mm., usually very flat, with a central mound, and then sinks into a pit, upon which it forms at first a very dense surface scum.

Gelatin stab; slight needle growth; a very shallow, dry pit is produced at first, in which liquid begins to collect after four or five days. Liquefaction produced very slowly in a horizontal layer.

Agar and potato; not characteristic.

Milk; is curdled at body heat in seven days, quite solid, and strongly alkaline. At room temperature it digests without curdling. The liquid is at first watery, soon becoming amber color, and later the color deepens, sometimes even to a *mahogany* color. When used for cream ripening no effect is produced on the butter.

No. 119. (Rare.) *Sarcina alba* (?)

Morphology; a sarcina form, $.6\mu$ to $.8\mu$ in diameter.

Gelatin plate; a slightly yellow, round, raised, opaque colony, which soon sinks into a pit. The pit remains clear, with a rough, granular nucleus.

Gelatin stab; a very narrow liquefying pit is produced, with a cloudy liquid. This broadens below the surface. Sometimes, however, a shallow, deep, dry pit is formed.

Agar and potato; a whitish, not abundant and not characteristic growth.

Milk; curdles in three days at 20° and in one day at 36°. Reaction amphoteric. Digestion of the curd very slight, or none. When used for ripening cream it produces a good flavored butter, with a good and typical butter aroma. One culture that I have regarded as the same produces a digestion of milk into a very slimy jelly.

No. 142. (Common.) *M. communis lactis*. (n. sp.)

Morphology; a *streptococcus*, with the elements .8 μ to 1 μ in diameter.

Gelatin plate; a round, brownish colony, slightly irregular in shape. Edge becomes after a couple of days somewhat lighter and surrounded by a liquefying pit, in which the granules spread irregularly. Occasionally a few blunt processes radiate from the center. The pit is rather large and at first is dry, subsequently liquefying.

Gelatin stab; a narrow funnel, with a dense, cloudy liquid is produced, with a deep, dry pit at the top. Below the air bubble the funnel spreads out into a turnip shape.

Agar; an almost snow white growth.

Potato; thin and watery, and spreading over the whole surface.

Milk; is curdled at 36° in three days, with amphoteric reaction. At room temperature curdles in three weeks. The curd is soft, with no whey, and the subsequent digestion is slight.

No. 147. (Common.) *M. liquefaciens acidi I*. (n. sp.)

Morphology; diameter, .7 μ to 1.1 μ , grouped in fours by dividing in two directions.

Gelatin plate; a densely granular liquid colony is produced, with a granular center, which is lobate or with folded edges, and which lies in a clear, liquefying pit. Later dense masses are formed outside of the center, and the whole pit becomes filled with irregular masses.

Gelatin stab; slow liquefier, producing a cloudy, shallow pit, with dense sediment. A scum with broken fragments appears. Even after three weeks gelatin is liquefied only for a quarter of an inch.

Agar and potato; a dry, snow white growth, quite opaque.

Milk; at 36° curdles in five days into a hard curd, with little whey. *Is acid in reaction, and has the smell of sour milk.* At room temperature the action is the same, although the acid is not quite so prominent. No digestion subsequently occurs, but a large amount of whey separates from the curd.

No. 168. *M. liquefaciens acidi II*. (n. sp.)

This agrees with No. 147 in many points, and may be the same. The following differences were noted:

Gelatin plate; a snow white bead, very opaque, is formed, growing to size of 2 mm., when it sinks into a slowly liquefying pit. Some colonies do not liquefy before the growth ceases.

Gelatin stab; a granular needle growth and a dense white surface on a slowly liquefying mass of gelatin.

Potato; growth is hardly visible.

Bouillon; becomes very cloudy.

Milk; is rendered acid, but the amount of acid is *insufficient to curdle* the milk unless it is heated.

These two cultures appear to me to be new, and their characteristics are so marked that I have ventured to give them a name.

No. 2. *M. acidi lactis*. (Krüger.)

Morphology; 1μ to 1.2μ in diameter.

Gelatin plate; at first a slight pit, which begins to liquefy, the colony being uniformly granular. The granules soon break up, distributing themselves through the pit, usually producing a nucleus, with a peripheral ring of granules. Outside the ring there may be a clear liquid outer zone. Eventually the whole becomes densely granular.

Agar; growth on the surface tends to become wrinkled, tenacious and sticky, and develops a yellowish or slight *salmon* color.

Potato; an abundant growth, somewhat *folded*, of a *flesh* or *salmon* color.

Milk is curdled at 20° or at 36° into a hard coagulum, with orange masses floating on the top. The reaction is *acid*. No digestion occurs. After a few days yellow lumps of fat frequently appear on the surface. Chemical analysis has shown butyric acid and alcohol to be present.

The last three organisms are peculiar in *liquefying gelatin*, but curdling milk with an *acid reaction*. This is unusual. Three or four such micrococci have been described before by Hueppe, Freudenreich, Krüger and Kozai. I have concluded that No. 2 may be the same as the species described by Krüger and Kozai.

GROUP VII. NON-LIQUEFYING, NON-CHROMOGENIC BACILLI.

This group, which is the most important group of dairy bacteria, is a very difficult one to arrange in any satisfactory manner. The different species are frequently very similar to each other, and the diagnostic characters difficult to determine. I am convinced that in some cases quite different bacilli are put under the same species because of difficulty in getting diagnostic characters for separating them.

I have found it most convenient to separate them, first in accordance with their power of producing an acid reaction in milk, and secondly in accordance with their morphological characters. A few are readily distinguished by their peculiar gelatin colonies, and some by their spore production.

Division A.

Bacilli producing an acid reaction in milk.

Nos. 125 and 89. *B. coli communis*.

This species of bacillus is extremely common in milk, although by no means universally found. It seems to show considerable variation. The two numbers above given are two of the many distinct cultures which I have identified with *B. coli*. They differ slightly. No. 125 shows gas bubbles in the gelatin

stab, while No. 89 does not, and No. 125 curdles milk more slowly, not curdling for two weeks, while No. 89 curdles in six days.

Both have been used for ripening cream, but produce butter with a markedly sour taste.

While this species is very common, it is rarely abundant enough to produce much influence upon the milk. It can therefore hardly be regarded as a distinctive dairy bacterium.

No. 208. (Extremely common.) *B. lactis aerogenes*. (?)

Probably identical with *B. acidi lactici* (Grotenfeld) and *B. a.* and *b.* of Guilleleau, and No. 8 of Eckels.

This is one of the most common species found in milk and cream. It is not only almost always found, but is also usually very abundant. Sometimes it appears to be the cause of the spontaneous souring of milk, since in some samples it is the only acid bacillus found. This is unusual, however, for though very common, it is usually far outnumbered by No. 206 and No. 202. It is also very variable in its physiological characters, as explained on page 24, but the many different cultures which I have studied have in general the following characters.

Morphology; size, $.7\mu$ by 1μ , with rounded ends.

Gelatin plate; deep colonies, opaque and oval. (Litmus gelatin turned very red.) Sometimes lobate, as if made up of many colonies. Surface colonies may be large (2 mm.), white, opaque beads, which may contain a gas bubble (dextrose gelatin). Sometimes they form projecting colonies, growing up from the surface of the gelatin to the height of 1.5 mm., though not more than .5 mm. in diameter. Sometimes they are like *B. coli* colonies, only more luxuriant.

Gelatin stab; abundant needle growth and a thick, white surface growth.

Agar; white, moist, glistening and semi-transparent. Abundant.

Potato; a creamy white, abundant, not folded growth. Moist.

Milk; at 20° rendered strongly acid, but commonly not curdling. Some cultures do curdle. At 35° curdles milk two to four days into a soft curd. In all cases the milk is strongly acid. There is a typical sour milk odor.

Fermentation tube; grows strongly in closed arm and in bulb, and produces much gas.

This includes Nos. 16, 53, 56, described in previous publications (Storrs Expt. Sta. Rep., 1890 and 1894).

The next two organisms belong to the *Typhosus* type (Fuller) which ferment milk sugar, but produce no gas.

No. 107. (Rare.)

Morphology; size, $.6\mu$ to $.8\mu$ by 1.3μ .

Gelatin plate; a thin, transparent, spreading colony, elevated into irregular ridges, becoming a centimeter in diameter, or larger.

Gelatin stab; an abundant needle growth, with a thin, transparent, spreading, irregular surface growth. *Hardly visible*.

Agar and potato; not characteristic.

Milk; not curdled at either 20° or 36°, but rendered acid, and will curdle when heated. Butter made from cream has a sour, clean taste, but not much flavor. A strong, but not typical aroma.

No. 137. (Rare.)

Morphology; size, .6 μ by 1.2 μ , variable.

Gelatin plate; spreading into a thin, transparent surface colony, which later becomes thicker and brownish or yellowish. It may even form a thick, yellowish bead, half a millimeter in diameter.

Gelatin stab; a good needle growth, white. On the surface an irregular, yellowish growth, spreading slightly and rather thick on the edge.

Agar; a dull yellow, transparent, but not abundant growth.

Potato; a thin, yellowish layer, which upon some moderately dry potatoes may be quite thick, but still yellowish.

Milk; sometimes not affected in the room, but in other cases is curdled in about two weeks into a soft, lumpy, incomplete curd. At 36° the curdling is more complete, but still soft, and the reaction is *amphoteric*. It has no effect upon butter.

This is the only non-liquefying organism found which curdles milk without rendering it acid.

The next two organisms differ from the others in producing spores.

No. 93. (Rare.)

Morphology; size, 1 μ by .6 μ , or in old cultures slightly larger. The old cultures show large oval spores, larger than the rods.

Gelatin plate; an irregular surface colony, streaked over the surface with irregular contorted lines. Grows to the size of 2 mm. on the surface. Colonies under the surface are lobed.

Gelatin stab; a good needle growth, with a thick, white, surface growth.

Agar; very thick and opaque and white. Later becoming somewhat yellowish.

Potato; very thick and transparent; of a whitish color, which later becomes dry and folded.

Milk; does not curdle at 20°, though will when heated. At 36° curdles in three days. Cream is rendered acid and sour, and butter made therefrom has rather an unpleasant, sour, cheesy taste.

No. 94. *B. ubiquitus lactis*. (?)

Morphology; size, .8 μ by 1.2 μ to 1.4 μ . In bouillon short chains are produced. A bipolar staining is shown, and spores are eventually produced in the middle of the rods. A non-staining capsule developed.

Gelatin plate; round, white, opaque colonies, with a dark center, frequently raised to form a bead.

Gelatin stab; abundant needle growth. Surface raised, white, forming a prominent nail head. The center is more elevated than the edge.

Agar; extremely irregular, spreading, thick, very white and smooth.

Potato; grows with extreme rapidity, forming a *transparent, white, glistening*, widely spreading growth, especially characteristic.

Milk; curdles in eleven to twelve days. Cream rendered acid and sour, and butter produced therefrom has especially good flavor, with no aroma.

I have with hesitation associated this with *B. ubiquitus* (Jordan). The differences between the two are considerable. Jordan does not describe any spore formation, and he states that *B. ubiquitus* curdles milk very rapidly, while No. 94 curdles it only after several days.

No. 206. *B. acidi lacti* I. (Esten.)

This organism, described in a previous Report of this Station (1896), must be regarded as the most important milk bacterium in the dairies of this vicinity. As described in that paper, it has been found almost universally in samples of milk from a very wide range of territory. It is by no means universally present, and if the milk from different cows be carefully studied separately, it is found that in many cases samples of milk are obtained with no specimens of this particular species present. But when mixed milk is studied it is found in almost all cases to contain this organism. Moreover, in the milk of ordinary dairies this organism forms the largest proportion of the bacteria present. In my studies of the bacteria of ripened cream it has been found that a proportion varying from 75% to 90% of the bacteria present in cream are of the species here described. It must, therefore, be looked upon as the dairy organism par excellence. Its description, though given elsewhere, may be for completeness sake best included here, and is as follows:

Morphology; short, plump rods, size, $.7\mu$ by 1.2μ . No chains are produced, and no spores are found.

Gelatin plate; in ordinary gelatin a small, finely granular colony produced, pearly white by reflected light, though slightly yellowish by transmitted light. In milk sugar gelatin rendered blue by litmus, the shape of the colony is characteristic and easily recognized. It is a round, opaque colony, the surface of which is always provided with *minute spines*. This spiny appearance is distinctly characteristic of this organism.

Gelatin stab; grows wholly below the surface as a rough, beaded needle track, with no surface.

Agar; no growth or a very thin, *almost invisible layer*.

Potato; growth on potato is scarcely visible.

Bouillon; becomes turbid and a sediment collects, but there is no scum, and no gas is produced.

Milk; is curdled in from six to twenty-four hours into a homogeneous jelly like curd, very hard, and containing no gas bubbles. There is no further change in the milk. It is intensely acid, and has a clear taste, with no odor.

This species is apparently identical with those described by Günther and Thierfelder, Leichmann, Weigmann and Kozai.

No. 202. *B. acidi lactici* II. (n. sp.)

Morphology; a short bacillus or coccus, $.7\mu$ by $.8\mu$.

Gelatin plate; an extremely small, clear, slightly yellowish colony is formed, never more than 1 mm. in diameter. It grows wholly under the surface or under a mica plate, but never on the surface.

Gelatin stab; grows along the needle track as a beaded, tolerably abundant growth, but no surface growth.

Agar and potato; there is no growth whatsoever upon these, nor is there any perceptible growth in bouillon. In a *fermentation tube* containing milk sugar bouillon there is no growth even in the closed arm.

Milk; is curdled in thirty-six hours to four days into a hard homogeneous curd, which is strongly acid. There is no separation of whey and no subsequent change.

This organism, next to the last, is the most common of our dairy species. It is found with practical universality in samples of mixed milk, and is present in very great numbers in ripened cream. While the number of this organism in ripened cream is not as great as in the case of the last species, the proportion is always high, and frequently reaches 20% in samples of typical ripened cream. This organism and the last comprise in many cases over 95% of the bacteria in the normally ripened cream, and they must therefore be regarded as the two most important dairy organisms in this region. *Neither of them produces the typical sour milk odor*, such as is developed by No. 208.

No. 197. *B. lactici aerobans*. (n. sp.)

This agrees with No. 202, except that it has no effect on milk, and its growth on agar is visible though very scanty.

Possibly these are the same as *Bacillus a.* of Freudenreich.

No. 41. (Originally found in milk from Uruguay.)

Morphology; a bacillus, occasionally clinging two together, and on potato frequently forming chains. Size, 1.1μ by $.6\mu$. When growing in potato it is slightly longer than in agar. No spores.

Temperature; grows best at about 20° – 23° C.; at 35° scarcely any growth; killed by temperature of 60° C. in ten minutes.

Gelatin plate; a smooth, round colony under surface. On surface a small, grey, raised bead-like colony, spreading somewhat, reaching size of 1 mm. occasionally. Not characteristic. After several years cultivation the gelatin colony was found to be always burr shaped, with irregular, more or less radiating margins.

Gelatin stab; slight needle growth. Spreads over surface as a moist, white, thick mound, forming a nail growth. Does not liquefy.

Agar; an abundant, white, smooth, glistening layer,

Potato; raised, thick, whitish or slightly yellow-tinged layer, differing in color with amount of moisture. When very moist, is white, but when dry tends to a yellowish tinge. Grows profusely. A pleasant *aromatic odor* developed.

Milk; does not curdle either at 20° or 35° . After two to three weeks becomes slightly translucent and brownish. The reaction is slightly acid. After three to four weeks it seems to digest into a translucent mass. It acquires a *pleasant aroma*.

This bacterium has been used widely for cream ripening, producing a pure flavored butter.

Division B.

Bacilli not rendering milk acid.

No. 126. (Rare.)

Morphology; a bacillus, size, $.7\mu$ by 1.2μ .

Gelatin plate; an opaque bead, 1 mm. in diameter, slightly scalloped edge. Very white, opaque.

Gelatin stab; typical nail growth. Not characteristic.

Agar; thick, white and abundant. The agar occasionally tinged green or yellow.

Potato; a yellowish or dirty white growth. Not characteristic.

Milk, no effect. Butter made from cream ripened with this organism has a cheesy aroma and taste.

No. 66. (Common.)

Agrees with No. 126, except that the milk is rendered slightly alkaline and the cheesy taste does not appear in the butter. Butter on the other hand develops an excellent nutty flavor of the highest quality.

Possibly these two organisms should be classified with the fluorescent, inasmuch as they occasionally render the agar slightly green.

No. 84. (Common.)

Morphology; size, $.6\mu$ by $.8\mu$ to 1.2μ . Occasionally six or eight unite together, but no long chains.

Gelatin plate; a rounded bead, with a smooth edge, finely granular and with a dark center. It spreads over the surface as a dry, thin growth, 2 mm. in diameter.

Gelatin stab; needle growth abundant. Surface growth dry, irregular and glistening.

Agar; widely diffused growth, branching irregularly and lobate. Yellow color.

Potato; growth thin and of a yellowish or brownish or even orange color.

Milk; no effect upon milk, cream or butter.

No. 198. (Common.) *B. communis lactis* II.

Morphology; size, $.8\mu$ by 1μ . Forms short chains in bouillon.

Gelatin plate; colony of the *B. coli* type. Surface colony spreads into a white, bluish growth, 1.5 mm. in diameter. They are slightly yellow under the microscope, and may have a somewhat raised center. The colony is moist and glistening.

Gelatin stab; abundant needle growth, with a thin, slightly spreading, white, dry, surface growth.

Agar; not characteristic.

Potato; a slight lemon yellow tinge, but not characteristic. No effect upon milk or cream.

Differs from No. 84 chiefly in the form of the *gelatin colony*. Probably the same as No. 26. Previously described. (Storrs Sta. Rep., 1893.)

194. (Very common.) *B. communis lactis* I. (n. sp.)

Morphology; size, $.6\mu$ by $.8\mu$.

Gelatin plate; a smooth, round, white colony, not very opaque, becoming 3 mm. in diameter, irregular and rough or lobate, or sometimes round, white, moist and regular.

Gelatin stab; needle growth abundant. A white, flat, glistening surface growth, which is first thin, but later becomes thicker and slightly yellow.

Agar; moderately thick, moist, smooth and transparent.

Potato; white, moist and thick.

Milk; no effect upon milk or cream.

This is very common in milk, though never in great numbers. It is the same as No. 55. Previously described. (Storrs Sta. Rep., 1893.)

No. 191. *B. radiata lactis*. (n. sp.) (Named also *B. citreus arborescens* on p. 43.)

Morphology; size, $.8\mu$ by 4μ . No long chains, but two or three may be united together. Surrounded by an unstained capsule.

Gelatin plate; widely spreading colony, with fine lines radiating from the center. Grows over the whole plate, with fibers permeating the gelatin. The fibers are knobbed. Fibers from two colonies may grow over the whole plate in the course of three days. To the naked eye the whole looks like a mould.

Gelatin stab; a slight needle growth, with a characteristic ground glass surface growth.

Agar; not characteristic.

Potato; a very thin growth, which may be slightly lemon yellow.

Milk; after three weeks is rendered slightly alkaline and semi-transparent.

This organism was found only once, and was unfortunately lost before its description was complete. Its very unique colony upon gelatin is, however, sufficient to distinguish it.

No. 74. (Rare.) *Proteus Zenkeri*. (?)

Morphology; size, 1μ by 2μ to 3μ , forming long threads in bouillon.

Gelatin plate; colonies especially characteristic. They start as round colonies, from which extend fine branches. These radiate widely and are at first fine and subsequently polypiform. Sometimes they are simply fine, radiating lines, not polypiform, and occasionally the colony is simply lobate, without radiating fibers.

Gelatin stab; along the needle track are lateral extensions forming thin sheets, thus producing the form of an inverted fir tree. Surface growth thin and irregular.

Agar; spreads rapidly from the needle track, with radiating fibers rather thick.

Potato; dirty white, brown, rough, with a sandy appearance.

Milk; no effect except a slight alkalinity. After three weeks it becomes slightly slimy. No effect on cream or butter made from the cream. Develops no aroma or taste.

No. 98. (Rare.)

Morphology; size, $.7\mu$ by 1.5μ to 2μ . Joining into chains.

Gelatin plate; a round, rough, granular colony, sometimes coarsely granular like a corn ball.

Gelatin stab; a moderate needle growth, with a thin, transparent, widely spreading, *hardly visible* surface.

Agar; moderately thick along the needle track, but with a thin, transparent, *hardly visible*, spreading edge growing over the agar.

Potato; rather scanty, but not characteristic.

Milk; no effect upon milk or cream. Butter develops a rather unusual aroma, but no flavor.

No. 12. (Rare.) *B. viscosus lactis* II. (n. sp.)

Morphology; a rod, four times as long as broad (size not measured), surrounded by a mass of slime.

Gelatin plate; a white bead, 1 mm. in diameter. Not characteristic.

Gelatin stab; abundant needle growth, with a thin, irregular rosette surface growth.

Agar; transparent and glassy, very thick and raised in irregular masses. The growth is extremely slimy, forming threads several inches long when lifted with the platinum loop.

Potato; growth greyish brown, mottled, abundant, tenacious, and slimy.

Milk; is rendered slimy and alkaline. Threads of a foot in length may be drawn from it with a platinum loop. An odor is developed in the milk, reminding one of *strong cheese*. After a month the milk becomes almost solid, although its sliminess has disappeared.

This bacillus appears to be similar in its general characters to *B. viscosus lactis* of Adamitz, but its morphology is quite different. While *B. viscosus* is nearly as broad as long, this No. 12 is a long, slender rod. It was isolated from milk in 1891, and has not been found since.

No. 25.

Morphology; a bacillus, $.7\mu$ by 2μ .

Gelatin plate; a minute, clear, round colony is produced, which is later raised into a bead, with concentric folds. It may spread to a diameter of 1 mm., and show a central nucleus with a dark outer rim. The edge may be rough and folded.

Gelatin stab; an abundant needle growth, which is rough and beaded. A slightly mounded surface growth, spreads widely and later is thin, transparent and dry. Color is white.

Agar; is white, moist and moderately thick.

Potato; is white or gray, dry and thick. Later it becomes yellowish and even brown.

The most convenient grouping of the liquefying bacilli has been found to be that adopted by Flügge, depending upon the character of the spore formation. It should be stated,

however, that less attention has been given to the liquefying bacteria than to the non-liquefying bacteria. While they are almost always present in milk, their relative number is always small in normal milk. The rapid growth of the numerous lactic bacteria commonly checks the multiplication of the liquefiers, so that they are always few in ordinary milk or cream. I am now convinced that they are of comparatively little importance in normal dairy processes. In ripened cream, as will be shown in a later paper, they are commonly only to be found in very small quantities. For these reasons less attention has been given to them in my investigations, and the list given below is therefore doubtless far from complete. The bacteria here listed doubtless include some of those described by Duclaux under the name of *Tyrothrix*, but the incompleteness of his description makes sure identification impossible. I have therefore not attempted to identify them with Duclaux's species.

GROUP VIII. LIQUEFYING BACILLI WITHOUT SPORES.

No. 200. (Rare.) *B. musci lactis*. (n. sp.)

Morphology; size, 1μ by 2μ to 5μ . Forms long chains, which look like strings of sausages. These form a tangled mass, forming a scum on gelatin.

Gelatin plate; a diffuse colony, 1 inch in diameter, made up of long fibers, growing chiefly under the surface of the gelatin, looking like a tuft of moss, thick in the center and gradually fading out around the edge. Quite characteristic network of fibers.

Gelatin stab; a ground glass, crumpled surface. Below surface there is a tree-like growth from the needle track, hardly visible. Liquefaction takes place slowly. There is eventually produced a liquid cone, with a central granular axis, shaped like an inverted cone. Eventually the liquefaction is complete.

Agar; widely spreading, with creeping branches on the surface like cotton threads. Eventually covering the whole surface.

Potato; growth is chiefly under the surface. The surface becomes rough and white and somewhat broken.

Bouillon; masses are formed floating in a clear liquid, and a scum appears later.

Milk; is curdled after three weeks, and becomes slowly digested into a translucent mass, full of flakes and showing a skin on the surface of a ground glass appearance.

No. 196. (Common) *B. varians lactis* I. (n. sp.)

Morphology; size, $.8\mu$ by 1.5μ , blunt ends.

Gelatin plate; a thin, spreading, transparent surface. When reaching a millimeter in diameter it sinks into a pit in a dense, granular mass. Sometimes

a few lobe-like shoots extend from the colony into the gelatin, *ending in prominent knobs*, quite characteristic.

Gelatin stab; a shallow cone produced. The gelatin then liquefying regularly into a dense, cloudy liquid.

Agar and potato; scanty growth, but not characteristic.

Milk; is curdled hard and rendered amphoteric, or sometimes acid. No digestion can be seen, but a watery whey subsequently deposits from a solid curd.

Nos. 176 and 139. *B. varians lactis* II. and III.

These two cultures I place with No 196. They agree in all points except the power of liquefying gelatin. No. 139 in gelatin stab forms a deep, dry pit, with no sign of liquid. No. 176 produces a dry pit, but later begins to liquefy at the bottom, and the liquefaction slowly deepens, while No 196, as shown, liquefies rapidly. The three were obtained from different localities and at different times.

They may be identical with *B. cloacae* (Jordan).

No. 64. (Rather common.) *B. circulans* II. (n. sp.)

Morphology; size, $.6\mu$ by 1.5μ . Long chains are produced in bouillon, but no spores found.

Gelatin plate; a granular bead is produced, which sinks into a dry pit. The pit liquefies, and the bacilli can be seen actively *circulating in the liquid*. Uniformly granular.

Gelatin stab; there is a growth along the needle track, producing a deep, narrow funnel, from which the liquid evaporates, so that there is a considerable portion of the funnel without liquid. A white sediment forms in the axis of the liquefying pit. The whole is peculiar and characteristic.

Agar; an abundant, yellowish growth. Not characteristic.

Potato; a somewhat thin, watery, transparent growth.

Milk; there is no curdling, but the milk digests into a weak alkaline liquid, which is cloudy and gives off unpleasant odors. Butter made from cream ripened with this organism develops a moderately good flavor and aroma. The putrefactive odor in the milk and cream is ordinarily not noticeable in the butter.

This is very similar to *B. circulans* of Jordan. I have, however, found no spores, while Jordan found them in most media. The growth on agar is also different. The peculiarities shown in the colony and the gelatin stab lead me to put it with the species described by Jordan as a *Variety II*.

No. 164. (Rare.)

Morphology; size, $.5\mu$ by $.8\mu$ to 1μ . Tapering ends and showing irregular stain.

Gelatin plate; a yellow colony in a deep pit. As liquefaction begins there is a curiously figured central lobate mass, with an outer clear zone.

Gelatin stab; a shallow, dry pit appears, which soon shows liquefaction at the bottom, an air bubble remaining for some time. Later liquefying over the whole surface into a cloudy liquid, with a dense sediment.

Agar; semi-transparent, thick growth.

Potato; moist, not very abundant growth, which may show an orange or brownish pigment.

Milk; is slowly digested without curdling into a red amber colored, watery liquid, with a jelly-like mass of undigested casein at the bottom. Is alkaline.

No. 129. (Rare.)

Morphology; size, $.8\mu$ by 1μ to 3μ .

Gelatin plate; colony at first irregular or round. It soon becomes rough and margined, and branches arise. It then develops into a liquefying colony, with a nucleus and radiating markings.

Gelatin stab; a slow liquefaction. There is a shallow pit, becoming a horizontal layer, of a cloudy liquid.

Agar; moist, white and thick. It spreads irregularly over the agar.

Potato; at first smooth, white and moist, but later becoming slightly yellowish and folded.

Milk; is curdled with an alkaline reaction and then slightly digested, the liquid becoming slimy.

No. 120. (Rare.) *Bacillus anana*. (n. sp.)

Morphology; size, $.5\mu$ by 1μ to 1.2μ .

Gelatin plate; a round, opaque, granular colony, breaking up to form a pit, covered with mottled, granular masses. There is frequently a nucleus and a zone of granular fragments.

Gelatin stab; a narrow pit, with a granular liquid. The pit broadens at the surface and contains very cloudy liquid. Later the whole gelatin is liquefied.

Agar; moist, white, abundant.

Potato; very thick, white and abundant, and having the odor of pine-apple.

Milk; curdles at 20° into a soft curd. No digestion noticeable. No curdling at 36° .

No. 68. (Rare.)

Morphology; size, $.6\mu$ by 1μ . Shows uneven stain and a capsule.

Gelatin plate; a pit forms, filled with irregular masses. It is frequently rosette formed at first, but breaks into opaque granules as the liquefaction begins.

Gelatin stab; a deep, dry pit formed, which later liquefies, a scum appearing on the liquid.

Agar; white, moist, thick at center of the inoculation line, but with a thin, scalloped edge. Later it becomes yellow.

Potato; a very profuse, abundant, moist, jelly-like growth covering the whole potato. May be white or yellowish. Very characteristic.

Milk; may curdle, or digest without curdling. Is alkaline. In about twelve days it becomes a nearly transparent liquid with a yellow scum. No effect on butter when used for ripening cream.

This is similar to No. 120, except for its action on milk.

No. 69. (Rare.)

Morphology; size, $.8\mu$ by 2μ .

Gelatin plate; a liquefying colony, with a nucleus and a broad, granular margin, or sometimes uniformly granular without the nucleus.

Gelatin stab; a narrow funnel, which widens as it liquefies into a broad funnel. The liquid has a granular tinge at the surface, and is cloudy, with a slight granular sediment.

Agar; rapid growth, spreading widely into a white, opaque layer, with irregular, glistening edge.

Potato; white, or cream white, and semi-transparent.

Milk; curdles in five to six days into a soft curd, which is alkaline. It then digests into a colorless liquid, with a bitter taste. Produces butter with a sharp, sour taste, but a thoroughly *typical butter aroma*.

GROUP IX. LIQUEFYING BACILLI WITH SPORES NO LARGER THAN THE ROD.

No. 207. *Bacillus subtilis*.

Not an uncommon inhabitant of milk.

No. 177. *Bacillus megatherium*.

This species has been found once or twice. It is easily recognized from its great size, 2.5μ , in diameter, and its spores of much less diameter. Its complete characters have not been studied here.

No. 184. (Rare.) *B. lactis* V. (?) (Flügge.)

Morphology; a large rod, with square ends forming long chains.

Gelatin plate; liquefies rapidly. Under the surface appear opaque, rough gelatin colonies, with a fibrous rim. Colonies liquefy rapidly, spreading into a uniformly granular or fibrous colony, 2 cm. in size in two days. When the colonies are near together the fibers become twisted and look like anthrax colonies.

Gelatin stab; needle growth abundant. There is a horizontal liquefaction, with a rough, white, wrinkled, tough *skin, looking like a mould*. The skin later becomes somewhat yellow, and the gelatin is finally completely liquefied, with a yellow scum.

Agar; abundant, rough, whitish yellow skin, with an irregular edge.

Potato; a very abundant growth, thick and dry, forming an almost *powdery white* surface.

Milk; no curd appears, but the milk digests in one to two weeks into a translucent liquid, with a thick, *folded scum* on the surface. It is strongly alkaline.

This appears to me to be quite similar to *B. lactis* V. (Flügge). It differs, so far as can be determined from his description, only in its action on milk, which, in Flügge's organism, did not produce the folded skin on the surface of the digested milk.

No. 145. (Rare.)

Morphology; size, 1μ by 3.5μ , blunt ends. Long chains occasionally produced. Spores spherical.

Gelatin plate; colony liquefies when it reaches the size of 1 mm., forming a dense, white layer, covering the whole surface of the liquefying pit.

Gelatin stab; liquefies at first as a small cone, and this slowly spreads over the whole surface, becoming covered with a dense, white film.

Agar; not characteristic.

Potato; very thick, dirty white layer, slightly transparent and gelatinous.

Milk; no effect upon milk either at 20° or 35° .

No. 114. (Rare.) *B. mesentericus fuscus*.

Morphology; size, $.8\mu$ by 2μ , forming long chains.

Gelatin plate; deep colonies are round, smooth and transparent. The surface colonies are raised, with a thin, transparent edge and irregular rim, creeping over the surface in irregular lobes. In five days it reaches the size of 1 mm., then it sinks into a liquefying pit, the colony remaining as a dense growth over the whole surface, with no clear liquid ring surrounding it.

Gelatin stab; needle growth abundant. A very shallow pit, with an air bubble in its center. The pit later spreads over the whole surface.

Agar; a somewhat dry, slightly folded growth.

Potato; a gray or yellowish, thick, highly folded skin.

Milk; at 20° does not curdle but digests slowly and becomes alkaline. At 26° curdles in six days and digests. Cream ripened with this organism produces butter with no aroma nor flavor.

GROUP X. BACILLI WHICH LIQUEFY GELATIN AND FORM SPORES LARGER THAN RODS.

These easily recognizable bacteria I divided into three divisions, according to the position of the spore.

Division A. Spores in the middle of the rod (spindle formed).

Division B. Spores at one end of the rod (tetanus type).

Division C. Two spores (?), one at each end of the rods.

Division A.

*Spores in the center of the rods.*No. 123. (Rather common.) *B. arborescens lactis*. (n. sp.)

Morphology; size, 1.8μ by 3μ , growing into long chains. Large spores produced of a size 1.2μ by 2μ , causing the rods to swell in the middle.

Gelatin plate; colony grows into a large felted ground glass mass, which sinks slowly in a shallow pit.

Gelatin stab; a thick, ground glass surface growth forms as a scum floating on a shallow funnel. This eventually becomes much folded. Later the whole becomes liquefied, and the scum remains folded and tenacious.

Agar; grows into remarkably spreading branching filamentous masses which cover the whole surface and even grow under the surface. Quite characteristic.

Potato; almost snow white, abundant growth which extends into the potato. The surface is raised into folded mounds.

Milk; rapidly curdled at 20° and at 35°, and digested into a cloudy, colorless or amber colored liquid with alkaline reaction. Butter made from cream ripened with this organism has an unpleasant flavor and aroma.

No. 154.

This organism is probably a variety of No. 123, differing only in growth on potato and in bouillon. The potato growth is scanty, the surface becomes covered with a slight, fuzzy growth, and later with small, rounded knobs containing spores. There is an abundant growth below the surface. In bouillon a jelly-like, tenacious, flocculent mass is formed in the liquid. In other respects this agrees with No. 123.

No. 181. (Rare.) *B. filiformis lactis*. (n. sp.)

Morphology; size, 1μ by 2μ . The rods have a thick capsule with a central staining body. Spores are 1.2μ by 1.8μ in size.

Gelatin plate; colony has a fine, granular center, breaking at its edge into a granular margin surrounded with a clear, liquefying zone. Later the margin shows *contorted lacing threads*.

Gelatin stab; a narrow, cylindrical liquefying funnel is formed, with a cloudy liquid. Much gas is produced showing *as bubbles on the surface of the liquid* as well as the gelatin. Sometimes, after long cultivation, it grows without producing this gas. Liquefaction becomes complete and a dense scum and sediment is formed.

Agar; may be dry and thin, but commonly forms a thick, widely spreading lobate or branching mass, which is dry, white and quite characteristic.

Potato; a moist, yellowish, slimy mass grows over the surface.

Milk; curdled in two days with little change in reaction. Cream is rendered slightly acid with a sour cream taste, and butter made therefrom has a good flavor, but no aroma.

No. 88. (Rare.)

Morphology; size, 1.5μ by 3μ . Chains of a dozen elements are formed and large spores produced in bouillon.

Gelatin plate; a liquefying pit filled with filaments. A central granular nucleus forms with coarse granular masses, which become uniformly distributed in the surrounding zone.

Gelatin stab; a simple pit is formed which at first contains no liquid, but later liquefaction occurs slowly.

Agar; yellowish or white, not characteristic.

Potato; decidedly lemon yellow at first and later becoming dry, wrinkled and quite yellow.

Milk; curdles at 20° in six days with a thick scum and a little whey. Is alkaline in reaction. Digests into an especially clear liquid with a tenacious scum and sediment. Cream develops an unpleasant odor and the fat separates as masses floating in the whey. Butter made therefrom has no taste nor aroma.

The following three organisms show considerable similarities, especially in their morphology. But the type of colony they produce in gelatin is so different that I cannot regard them as identical. They resemble somewhat the

B. mycoides of Flügge, although each shows distinct points of difference from Flügge's organism. I have therefore named them *B. mycoides lactis* I., II., III.

No. 102. (Common.) *B. mycoides lactis* I. (n. sp.)

Morphology; 1μ by 2μ in length, forming long chains with oval spores.

Gelatin plate; deep colonies are at first irregular and show irregular radiating fibers. *A proteus-like colony*. The surface colonies form a pit with a large nucleus and a loosely granular outer zone. Liquefaction slowly extends.

Gelatin stab; a narrow funnel broadening at the top is formed, with a dense sediment. It liquefies then over the surface of the gelatin, and the liquefaction deepens uniformly with a very granular liquid containing a dense white sediment.

Agar; growth is tough and dry and broken easily into fragments. Later becomes very white from being covered with spores.

Potato; dry and rough and even, becoming snow white.

Milk; curdles in two days at 36° into a soft curd, faintly alkaline. Curdles also at 20° . Digests slowly producing a rancid odor. The digested liquid is colorless, though when the digestion is complete the liquid may be amber colored. It is without effect upon the flavor or aroma of butter.

No. 124. (Common.) *B. mycoides lactis* II. (n. sp.)

Morphology; size, 1μ by 2μ . Grows into long threads. The individual elements show square ends. A capsule is developed and large spores are prominent.

Gelatin plate; a pit is formed in which a tangled mass of threads is formed very much like anthrax colonies. It is quite characteristic. Becomes half a millimeter in diameter and then liquefies.

Gelatin stab; a shallow funnel is produced which liquefies in a horizontal layer. The liquid is clear with a dense scum and a dense sediment.

Agar; a tough, white growth is formed, readily breaking into lumps.

Potato; growth thin and dry, almost snow white, commonly a putty-like texture.

Milk; curdles rapidly at 36° in one day and at 20° in a week. Digests into a cloudy liquid which is colorless or amber colored.

No. 111. *B. mycoides lactis* III. (n. sp.)

Agrees with No. 124 except in the following points:

Gelatin stab; peculiar radiating growth arises from the needle track. This is unlike the common "fir tree" type, inasmuch as the radiating growths are in branches and are as long at the top as at the bottom of the gelatin. They grow moreover obliquely rather than horizontally. Later the liquefaction begins and is finally complete.

Potato; this grows into a dry, velvety, spreading growth all over the surface. Later becomes very snow white and dry.

No. 138. (Rare).

Morphology; size, 1μ by 2μ to 3μ . Forming long threads in bouillon. Rods with square ends.

Gelatin plate; brownish granular colonies with an irregular edge, which become surrounded by a liquefying pit. The pit is finely granular with a nucleus. A nucleus soon breaks up into variously formed irregular masses and the whole colony becomes uniformly granular.

Agar; not characteristic.

Potato; much folded into thick, *contorted folds*, with a yellowish tinge.

Milk; the milk is, after several days, curdled into a soft curd, which digests at once into a colorless liquid, completely dissolving the casein. Cream ripened by this organism develops a very unpleasant flavor and aroma in the butter.

No. 150. (Uncommon.)

Morphology; size, $.7\mu$ by 1.3μ . Long threads of rods with rounded ends.

Gelatin plate; colonies 2 mm. in diameter forming thick, raised, yellowish masses lying in a broad pit. Liquefaction proceeds slowly; the colony remains as a flat, dense, opaque mass, folded and wrinkled. Later a peculiar *snow white mass arises in the center of the colony* looking like a mould. This is extremely peculiar and very characteristic.

Gelatin stab; a shallow pit is produced. The growth is slow, and a clear liquid is formed with a dense, yellow scum, but no sediment. Later the liquid becomes cloudy and a sediment collects which may be white or yellow.

Agar; not characteristic.

Potato; develops a wrinkled, brown skin, which later becomes dry and highly folded.

Milk; is curdled in four days at 36° and in two weeks at 20° , into a soft alkaline curd. It eventually digests into a somewhat transparent mass, but the digestion is incomplete. A very pleasant odor is developed.

No. 51. (Rare.)

Morphology; size, $.8\mu$ by 1.5μ to 2μ . Rods with square ends.

Gelatin plate; colony irregular and developing a peculiar, characteristic, contorted mass arranged in parallel rows of thread. *A proteus-like colony*.

Gelatin stab; growth is slow, and a shallow pit is formed which in about three weeks half liquefies the gelatin, but remains to the end as a cone shaped pit.

Agar; not characteristic.

Potato; a velvety white, even *snow white*, thick growth.

Milk; curdles in two days into a soft, jelly-like mass which rapidly digests into a yellowish, cloudy liquid which is alkaline. Butter made from cream ripened with this organism has but little taste and what taste it has is unpleasant.

No. 153. (Not common.)

Morphology; size, 1.2μ by 3μ to 6μ , with blunt ends. Threads not long and frequently broken.

Gelatin plate; a large colony, 1 cm. in diameter, uniformly granular, with a radiating rim.

Gelatin stab; a deep funnel formed with liquid at bottom and an air space at the top. The liquefaction increases and a broad cone of slightly cloudy liquid is produced with a scum. Later the liquid becomes clear and the scum dense.

Agar; white, opaque and moderately thick. The growth is tough and not easily broken into fragments.

Potato; a ground glass, much folded layer is produced with liquid under the folds. Later the folds increase and the whole becomes white.

Milk; curdles rapidly at 20° and 36° into a hard curd and no whey. Alkaline. Digests into a clear, yellowish liquid.

Division B.

Spores at one end of the rod.

No. 189. (Rare.) *B. arborescens lactis* II. (n. sp.)

Morphology; size, .8 μ by 4 μ . The spore is 1 μ by 1.2 μ . Occasionally two or three rods together, but no chains.

Gelatin plate; colonies become 1 cm. in size or larger, showing radiating fibers strewn with knots. The fibers themselves are fine and branching. The knots look like isolated colonies and each frequently shows secondary radiation. Fibers grow mostly under the surface. Very characteristic.

Gelatin stab; an arborescent growth underneath the surface of the gelatin. Branches extending horizontally from the needle track and ending in knobs. In two days a dry pit is formed on the surface with numerous disjointed colonies extending from this pit to the edge of the tube. Later liquefies at the surface with a dense white cloudy liquid.

Agar; surface curdled completely with a thin, hardly visible growth.

Potato; thin and scanty growth.

Bouillon; a tough scum is formed which sinks while disturbed and forms a flocculent sediment. Later the scum sinks and the liquid is slightly cloudy.

Milk; no effect produced upon milk.

Division C.

Bacilli with two spores, one in either end.

The following species is certainly peculiar. The rods are very long, from 2 μ to 6 μ , and in each end of the rods may frequently be seen a clear unstained body. I have regarded them as spores, even though it has generally been thought that a bacillus with two spores has not been found. At all events this species is very unlike any other found and may be most easily recognized from this apparent double spore formation. I have therefore placed it in a division by itself with a distinct specific name.

No. 190. (Rare.) *B. dispora lactis*. (n. sp.)

Morphology; size, 1.8 μ by 2 μ to 6 μ . Spores are about 1 μ to 1.5 μ , variable in size. Long chains of threads are formed with rounded or tapering ends, like a string of sausages.

Gelatin plate; a round, tough colony is produced, yellowish in color, easily removed intact by a platinum loop. When reaching the size of 1 mm. it sinks into a slowly liquefying pit, but the mass of bacilli remain as a distinct nucleus for a long time, the liquid being clear.

Gelatin stab; a shallow funnel is formed and the liquefaction becomes complete.

Agar and potato; not characteristic except that both show a tendency to have isolated clumps rather than a uniform layer.

Milk; at 20° is curdled and rendered amphoteric or alkaline. A slight digestion is apparent.

The following list is an index to the pages on which the different organisms are described. The species which I have named or have identified with species named by other investigators are given by name as well as by number; the others by number only. In accordance with general usage the new species are indicated by the abbreviation n. sp.:

	PAGE.		PAGE.
2. <i>M. acidi lactis</i> , - - -	49	93. - - - - -	51
5. <i>B. viscosus</i> , - - -	33	94. <i>B. ubiquitus lactis</i> (n. sp.), -	51
12. <i>B. viscosus lactis</i> II. (n. sp.),	56	98. - - - - -	56
16. <i>B. lactis aerogenes</i> , - - -	50	100. <i>B. aureus lactis</i> II. (n. sp.),	39
21. <i>B. fluorescens schuykilliensis</i> , - - -	32	102. <i>B. mycoides lactis</i> I. (n. sp.),	63
25. - - - - -	56	103. <i>B. aureus minutissimus</i> (n. sp.), - - -	36
26. - - - - -	54	104. <i>M. varians lactis</i> (n. sp.), -	37
31. <i>B. fluorescens liquefaciens</i> ,	32	105. - - - - -	41
37. - - - - -	47	107. - - - - -	50
41. - - - - -	53	109. - - - - -	47
42. <i>M. rosaceus lactis</i> (n. sp.),	34	111. <i>B. mycoides lactis</i> III. (n. sp.), - - -	63
47. - - - - -	46	113. <i>M. varians lactis</i> (n. sp.), -	37
48. <i>B. lactis erythrogenes</i> , -	39	114. <i>B. mesentericus fuscus</i> , -	61
51. - - - - -	64	115. <i>B. ruber lactis</i> (n. sp.), -	35
53. <i>B. lactis aerogenes</i> , - -	50	116. <i>B. lactis erythrogenes</i> II. (n. sp.), - - -	40
55. <i>B. communis lactis</i> (n. sp.),	55	117. <i>M. citreus lactis</i> (n. sp.), -	40
56. <i>B. lactis aerogenes</i> , - -	50	118. <i>M. giganteus lactis</i> (n. sp.),	46
58. <i>M. acidi lactici</i> III., - -	44	119. <i>Sarcina alba</i> , - - -	47
60. <i>M. acidi lactici</i> I., - -	43	120. <i>B. anana</i> , - - -	59
62. <i>M. rubidis lactis</i> (n. sp.), -	34	121. <i>M. arborescens lactis</i> (n. sp.),	46
64. <i>B. circulans</i> II. (n. sp.), -	58	123. <i>B. arborescens lactis</i> (n. sp.),	61
66. - - - - -	54	124. <i>B. mycoides lactis</i> II. (n. sp.),	63
68. - - - - -	59	125. <i>B. coli communis</i> , - - -	49
69. - - - - -	60	126. - - - - -	54
70. - - - - -	45	128. <i>B. fluorescens minutissimus</i> ,	32
72. - - - - -	41	129. - - - - -	59
74. <i>Proteus Zenkeri</i> , - - -	55	130. <i>M. viscosus lactis</i> (n. sp.), -	44
75. - - - - -	45	131. <i>B. filiformis lactis</i> (n. sp.),	62
78. <i>M. acidi lactici</i> II., - 39,	44	137. - - - - -	39, 51
80. - - - - -	45	138. - - - - -	63
82. <i>B. fluorescens non-liquefaciens</i> , - - -	33	139. <i>B. varians lactis</i> III., -	58
84. - - - - -	54	141. - - - - -	38, 43
85. - - - - -	46	142. <i>M. communis lactis</i> (n. sp.),	48
88. - - - - -	62	145. - - - - -	61
89. <i>B. coli communis</i> , - - -	49	147. <i>M. liquefaciens acidi</i> I. (n. sp.), - - -	48
90. <i>B. fluorescens non-liquefaciens</i> , - - -	33	149. <i>B. citreus lactis</i> I. (n. sp.),	42
91. <i>B. citreus acidi</i> (n. sp.), -	41		

	PAGE.		PAGE.
150. - - - - -	64	188. <i>M. aureus lactis</i> , - - -	36
151. - - - - -	35	189. <i>B. arborescens lactis</i> II. (n. sp.), - - -	65
153. - - - - -	64	190. <i>B. dispersa lactis</i> (n. sp.), -	65
154. <i>B. arborescens lactis</i> (n. sp.),	62	191. <i>B. radiata lactis</i> (n. sp.), -	55
159. - - - - -	37	194. <i>B. communis lactis</i> I. (n. sp.),	55
161. <i>B. citreus lactis</i> II. (n. sp.),	42	196. <i>B. varians lactis</i> I. (n. sp.),	57
162. - - - - -	38, 43	197. <i>B. lactici aerobans</i> (n. sp.),	53
164. - - - - -	58	198. <i>B. communis lactis</i> II., -	54
167. <i>M. citreus lactis</i> (n. sp.),	41, 46	199. <i>Sarcina flava</i> , - - -	36, 40
168. <i>M. liquefaciens acidii</i> II. (n. sp.), - - -	44, 48	200. <i>B. musci lactis</i> (n. sp.), -	57
169. - - - - -	38	201. <i>Sarcina lutea</i> , - - -	40
170. <i>B. aureus acidii</i> (n. sp.), -	38	202. <i>B. acidii lactici</i> II. (n. sp.),	52
174. - - - - -	40	205. <i>B. aureus lactis</i> (n. sp.), -	38
176. <i>B. varians lactis</i> II., -	58	206. <i>B. acidii lactici</i> I., - - -	52
177. <i>B. megatherium</i> , - - -	60	207. <i>B. subtilis</i> , - - -	60
184. <i>B. lactis</i> V., - - -	60	208. <i>B. lactis aerogenes</i> , - - -	50
186. - - - - -	45	209. <i>B. prodigiosus</i> , - - -	34
187. - - - - -	42		

REFERENCES.

List of references of the most important articles upon systematic bacteria with special reference to dairy species.

Adametz, - - -	Landw. Jahrb., - - -	1889
" - - -	Oesterr. Monatschr. f. Thierh., - - -	1890
Bockhout, - - -	Over lange Wei, Delft., - - -	1897
Chester, - - -	Delaware Sta. Rep., - - -	1897
" - - -	Delaware Sta. Rep., - - -	1898
Claus, - - -	Inaug. Dis. Wurzburg, - - -	1889
Conn, - - -	Storrs Sta. Rep., - - -	1893
" - - -	Storrs Sta. Rep., - - -	1894
" - - -	Centbl. f. Bact. u. Par., II., V., - - -	1899
Duclaux, - - -	Le Lait, Paris, - - -	1887
Dyar, - - -	N. Y. Acad. of Sci., VIII., - - -	-
Eckles, - - -	Centbl. f. Bact. u. Par., II., IV., - - -	1898
Enmerling, - - -	Centbl. f. Bact. u. Par., II., IV., - - -	1898
Epstein, - - -	Arch. f. Hyg., XXXVII., - - -	1900
Esten, - - -	Storrs Sta. Rep., - - -	1896
Flügge, - - -	Die Microorganismen, Leipzig, - - -	1896
" - - -	Ztschr. f. Hyg., XVII., - - -	1894
v. Freudenreich, - - -	Ann. d. Microg., - - -	1890
" - - -	Centbl. f. Bact. u. Par., II., III., - - -	1897
" - - -	Centbl. f. Bact. u. Par., II., IV., - - -	1898
" - - -	Centbl. f. Bact. u. Par., II., V., - - -	1899
" - - -	Landw. Jahrb. der Schweiz., - - -	1893
" - - -	Landw. Jahrb. der Schweiz., - - -	1894

v. Freudenreich,	-	-	Landw. Jahrb. der Schweiz.,	-	-	-	1896
Frankland,	-	-	Ztschr. f. Hyg., VI.,	-	-	-	1889
Fuller and Johnson,	-	-	Jour. Exp. Med.,	-	-	-	1899
Gessard,	-	-	De la pyocyanie et de son microbe, Paris,	-	-	-	1892
Gosio,	-	-	Arch. f. Hyg., XXI.,	-	-	-	1894
Grotenfelt,	-	-	Fortschr. d. Med.,	-	-	-	1889
Guilleleau,	-	-	Landw. Jahrb. der Schweiz.,	-	-	-	1890
"	-	-	Ann. d. Microg.,	-	-	-	1892
Günther and Thierfelder,	-	-	Arch. f. Hyg., XXV.,	-	-	-	1895
Keferstein,	-	-	Centbl. f. Bact. u. Par., I., XXI.,	-	-	-	1897
Henerci,	-	-	Inaug. Dis., Basel,	-	-	-	1893
Hueppe,	-	-	Mitth. a. d. k. Gsudhtsamte,	-	-	-	1884
Jordan,	-	-	Rep. of Mass. St. Bd. of Health,	-	-	-	1890
Kozai,	-	-	Ztschr. f. Hyg., XXXI.,	-	-	-	1899
Kramer,	-	-	Monatsh. f. Chemie,	-	-	-	1889
Krüger,	-	-	Centbl. f. Bact. u. Par., VII.,	-	-	-	1890
Kuprianow,	-	-	Arch. f. Hyg., XIX.,	-	-	-	1893
Leichmann,	-	-	Landw. Vers. Stat., XLIII.,	-	-	-	1894
"	-	-	Milch Ztg.,	-	-	-	1894
"	-	-	Milch Ztg.,	-	-	-	1896
"	-	-	Centbl. f. Bact. u. Par., II., II.,	-	-	-	1896
"	-	-	Centbl. f. Bact. u. Par., II., V.,	-	-	-	1899
Leichmann and Bazarewski,	-	-	Centbl. f. Bact. u. Par., II., VI.,	-	-	-	1900
Loeffler,	-	-	Berl. klin. Wchnschr.,	-	-	-	1887
Marpmanh,	-	-	Ztschr. f. ang. Mikr.,	-	-	-	1896
"	-	-	Ergnznghsht. z. Cent. f. allg. Gsudhtspflg, II.,	-	-	-	1886
Migula,	-	-	System der Bakterien, Leipzig,	-	-	-	1890
Nenki and Sieber,	-	-	Monatsh. f. Chem.,	-	-	-	1890
Pammel,	-	-	Iowa Sta., Bul. 21,	-	-	-	1893
Ratz,	-	-	Arch. f. wissensch. u. prakt. Thierh., XVI.,	-	-	-	1890
Schardinger,	-	-	Monatsh. f. chem.,	-	-	-	1890
Schattenfroh & Grassberger,	-	-	Centbl. f. Bact. u. Par., II., V.,	-	-	-	1899
Tate,	-	-	Jour. Chem. Soc. Trans.,	-	-	-	1893
Troili-Peterson,	-	-	Ztschr. f. Hyg., XXXII.,	-	-	-	1897
Unna Tomanasoli,	-	-	Monatsh. f. prakt. Dermat., IX.,	-	-	-	-
Ward,	-	-	Cornell Sta., Bul. 165,	-	-	-	1896
Weigmann,	-	-	Milch Ztg.,	-	-	-	1889
"	-	-	Landw. Wochenbl. f. Schleswig-Holstein,	-	-	-	1890
"	-	-	Milch Ztg.,	-	-	-	1893
"	-	-	Milch Ztg.,	-	-	-	1896
"	-	-	Centbl. f. Bact. u. Par., II., V.,	-	-	-	1899
Winkler,	-	-	Centbl. f. Bact. u. Par., II., I.,	-	-	-	1895
Wright,	-	-	Mem. Nat. Acad. Sci.,	-	-	-	1895
Zangmeister,	-	-	Centbl. f. Bact. u. Par., XVIII.,	-	-	-	1896